



ChromoQuant®

Visualizer v2.2

User's Guide

Decision support software for analysis
of ChromoQuant QF-PCR samples



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Contact and Support

Information and downloads can be accessed from CyberGene's website (www.cybergene.com) or www.chromoquant.com

Support requests can be sent to chromoquant@cybergene.se or through your local distributor.

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1 Introduction

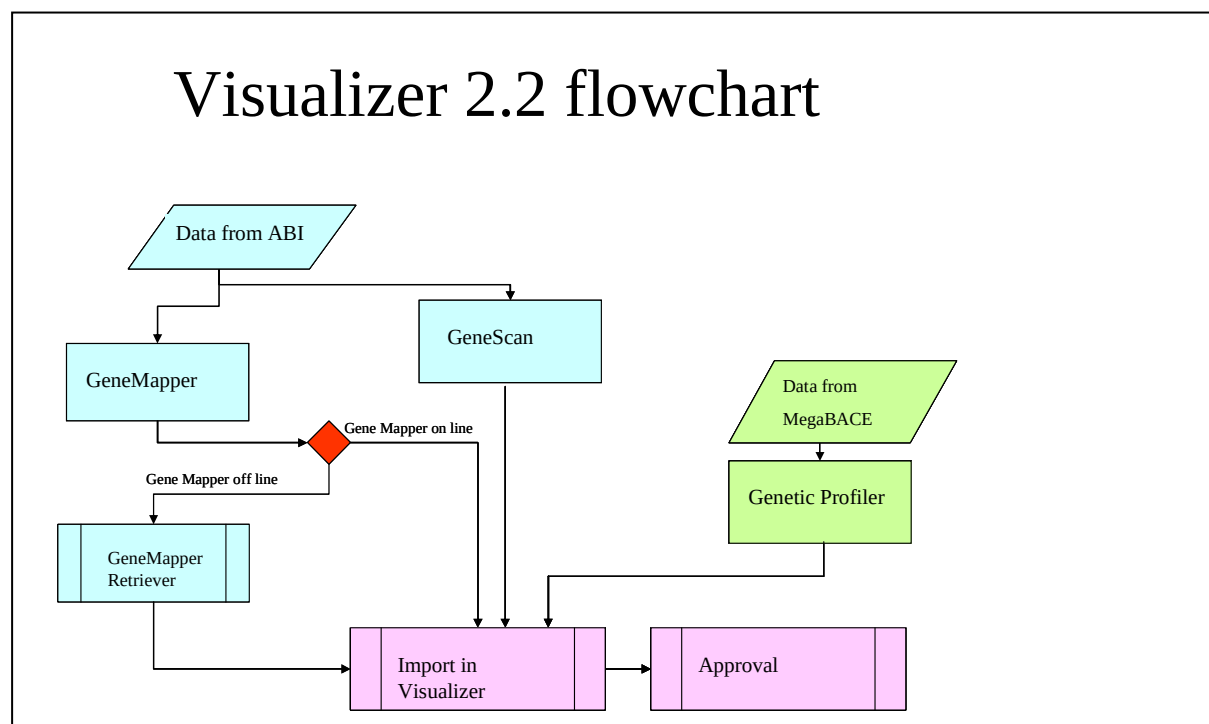
This manual describes how to handle the ChromoQuant Visualizer software. Visualizer interprets QF-PCR data in accordance with the *Best Practices Guidelines* issued by Association for Clinical Cytogenetics¹. Appendix 1 contains some additional guidelines for interpreting QF-PCR results.

Visualizer is a not only a software for interpreting results from prenatal testing of the most common chromosomal disorders, i.e. Down syndrome, Patau syndrome, Edward syndrome, Klinefelter syndrome and other sex chromosome aneuploidies, it is also a comprehensive Laboratory Information Management System (LIMS). The software will function as a database and a qualified decision support system for ChromoQuant QF-PCR results generated in your laboratory.

The sample files must be imported from your DNA analysis software, e.g. GeneMapper (ABI) or Genetic Profiler (MegaBACE).

The Visualizer software is compatible exclusively with data generated by the ChromoQuant QF-PCR kit, produced by CyberGene AB.

For handling of CyberGene's ChromoQuant kits and principles for interpreting results please refer to each kit's respective *Instructions For Use* (IFU) and to the *ChromoQuant User's Guide*.



¹ http://www.cytogenetics.org.uk/prof_standards/acc_cmgs_qfpcr_bp_dec2007_2.01.pdf

1.1 Installing the Visualizer Software

Please refer to the separate Installation Manual.

2 Sample naming

In order for Visualizer to be able to analyze the samples properly they must be given names according to a standard. This is the first step to take, before entering into Visualizer. If, however, the names are not given according to this standard, the software will prompt you to alter the names before importing the samples for analysis.

The sample name is made up of three sections. First the unique sample id (usually a lab number), secondly the panel identifier (13-18, 21, 13E, 18E, 21E or XYE) and thirdly a comment. A delimiter character “#” separates the groups. The delimiter is unique and must not be used anywhere else in the sample name.

For example, let us say that the lab number is *patient-123*. For this patient you prepare two PCR reactions using the ChromoQuant 311 kit. When running the fragment analysis you give the samples’ name in the sample sheet, in this case:

```
patient-123#13-18#any-text  
patient-123#21#any-text
```

```
Samles run with extra markers:  
patient-123#13E#any-text  
patient-123#18E#any-text  
patient-123#21E#any-text
```

You can alter delimiter character and panel identifier in Visualizer’s settings that you can open in the *Tools -> Settings* menu, see Settings section. In the settings window select the sample tab:

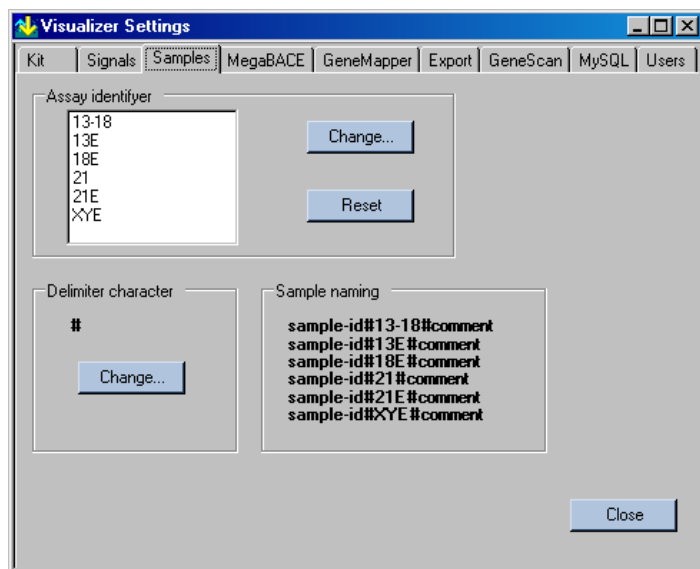


Fig 1: Visualizer’s setting window where sample naming can be customized.

When using the 412 or extra marker kits only one PCR reaction is needed. When running the fragment analysis you give the sample’s name in the sample sheet, in this case:

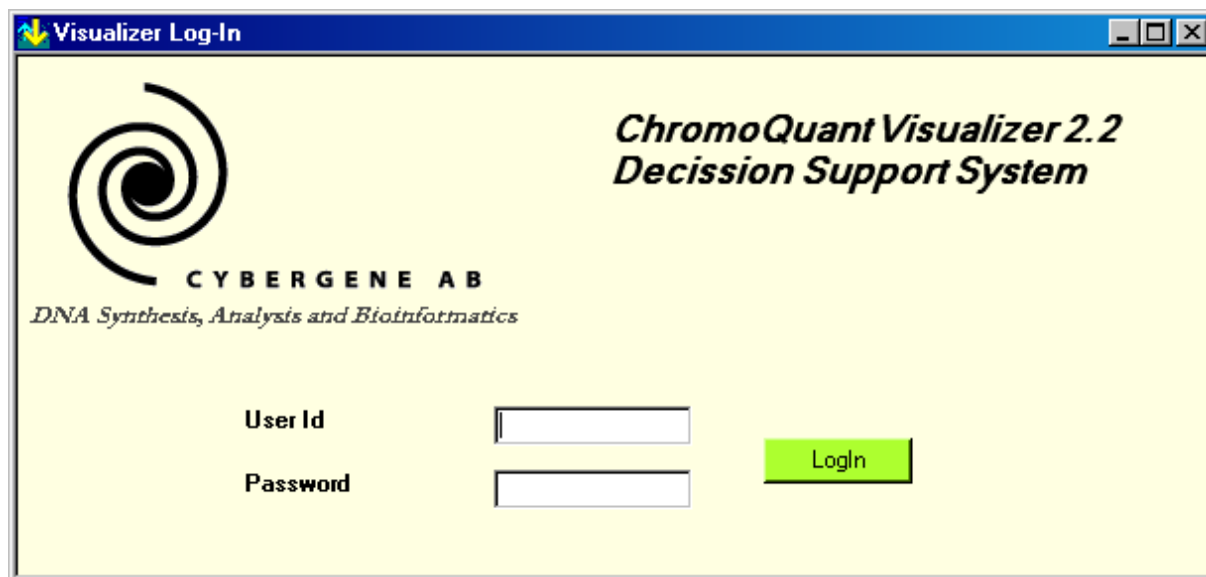
```
patient-123#13-18-21#any-text  
patient-123#13E#any-text  
patient-123#18E#any-text  
patient-123#21E#any-text
```

Note: The sample name, in this case the patient number, ensures that extra markers, when needed, are identified to the same sample collection as the main kit.

Visualizer will group all samples with the same identity. In this case it will recognize that the four tests above belong together. The panel identifier tells Visualizer which markers to analyze.

3 Open and Close Visualizer

When you start Visualizer, either from the desktop icon or from the Program menu, a login window first appears.



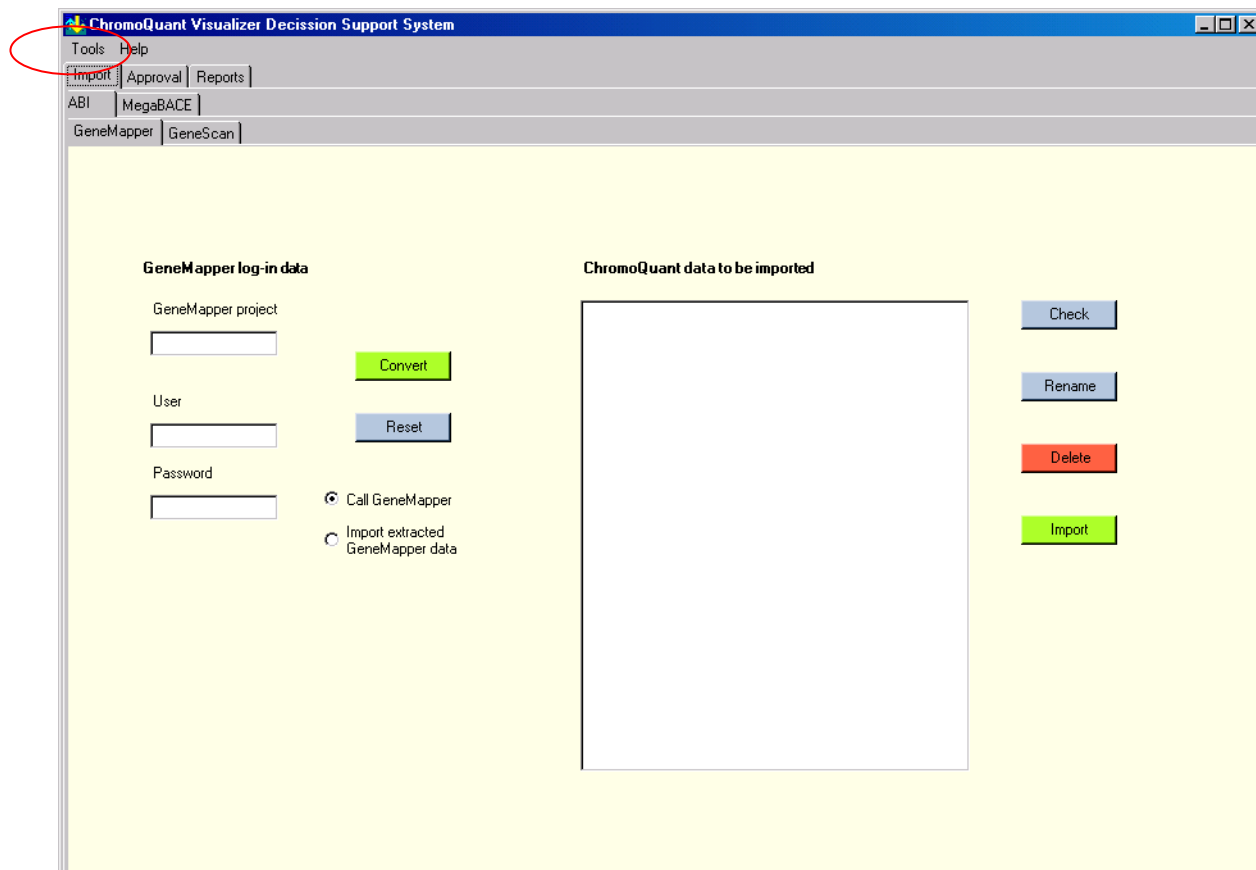
Enter your login details and click the *Login* button. The default user is Administrator. This user has User Id: **admin** and Password: **admin**.

We recommend that you create unique user accounts for each user of Visualizer. For details about user accounts see "Setting user properties" part under the Settings section.

In order to close Visualizer select *Tools -> Exit* from the menu.

4 Settings

To alter or review your Visualizer settings select *Tools -> Settings* from the menu. This action will open up the settings window.



There are several settings that can be applied to Visualizer. The settings have been divided into categories that can be accessed by selecting the appropriate tab. The following sections contain detailed descriptions of each setting category.

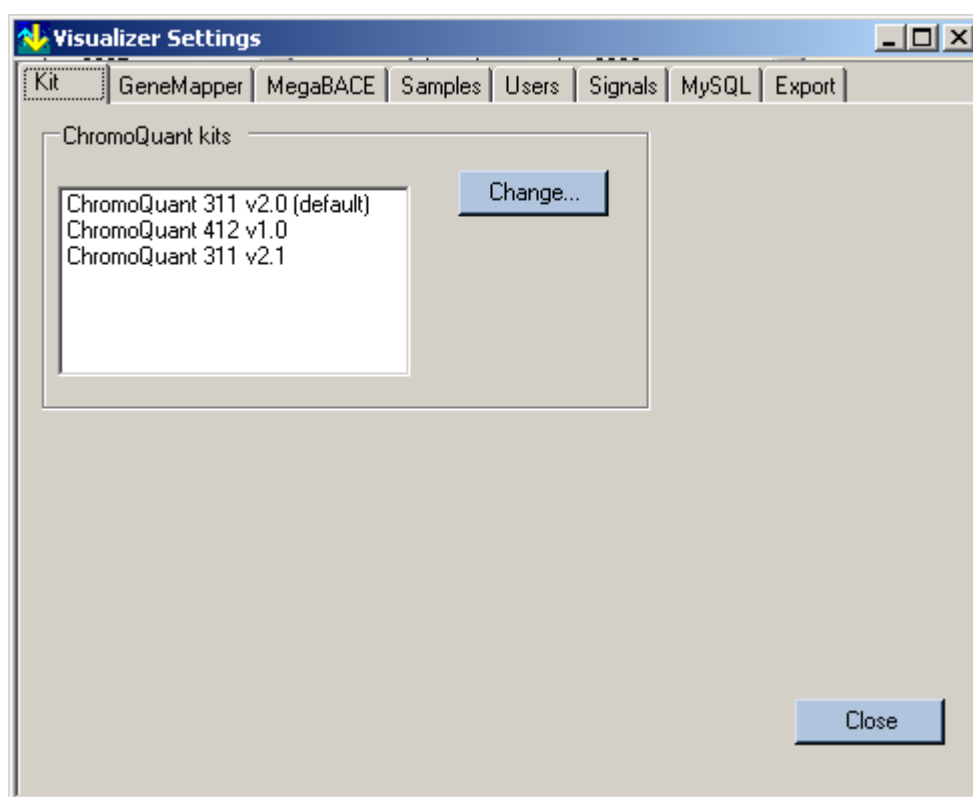
4.1 Setting default ChromoQuant kit

Tools -> Settings -> Kit

Each user has to define a default ChromoQuant kit, normally used in their laboratory. This will determine naming convention, genotyping and report generation. If you are working with several ChromoQuant kits then you have to alter this setting when alternating between data for each respective kit.

When changing the default setting just select the new default kit and click the *Change* button. The listing of ChromoQuant kits will be updated with the new default. The change is immediate and will affect new imports, genotyping and reports without having to restart Visualizer.

Note: Different Visualizer users can have different default ChromoQuant kits.

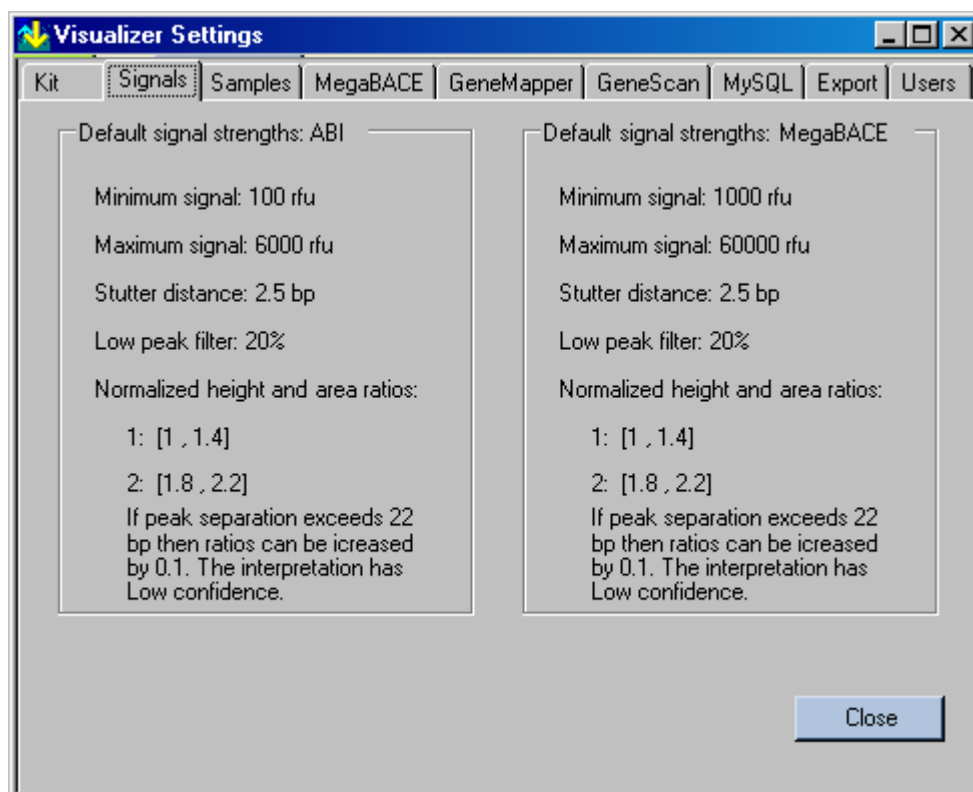


4.2 Reviewing instrument and fragment signal settings

Tools -> Settings -> Signals

This setting allows you to review signal strength and filter settings for each respective instrument type.

Settings for interpreting peak ratios are also presented herein. Any user cannot update the Signals section. This is a conscious design decision. Any genotyping done by Visualizer will thereby be in strict accordance with the QF-PCR best practices guidelines. Data that fail to comply with these guidelines, for any reason, should be re-run as a precaution.



Note: The ABI maximum signal, 6000 rfu, applies to all ABI sequencers with the exception of ABI 3730 that accept higher signals. However, Visualizer can analyze 3730 data although indicated overflow must be disregarded.

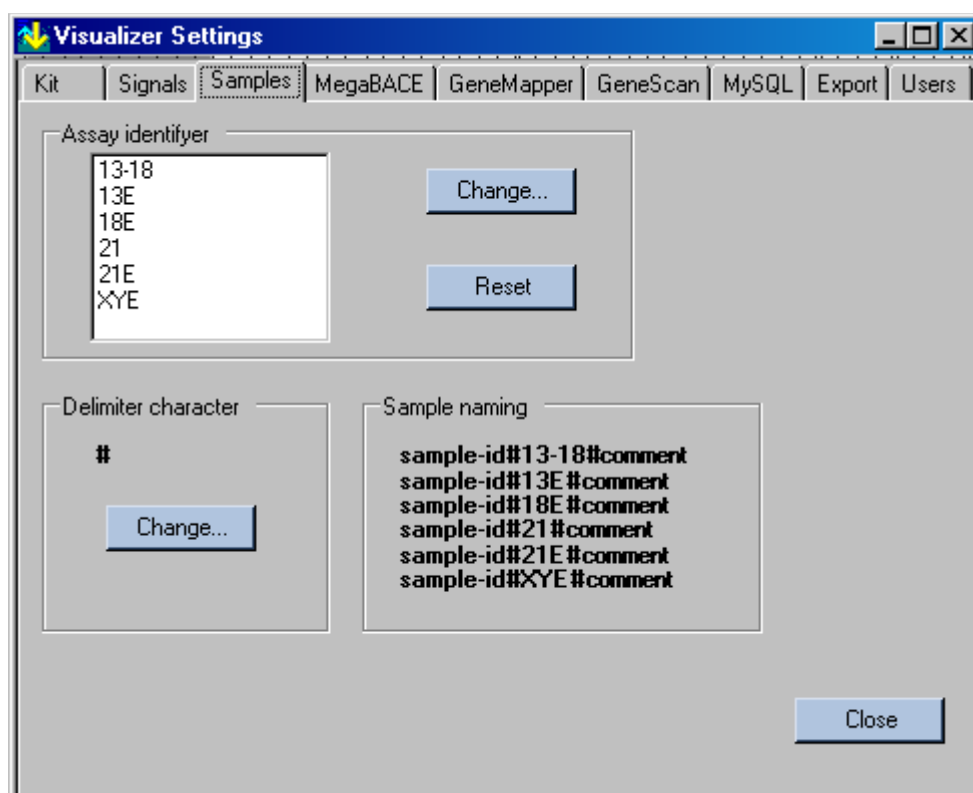
4.3 Setting sample naming

Tools -> Settings -> Samples

For naming conventions see the section covering *Sample naming*.

If you prefer to use a non-default delimiter or assay identifier then you can make appropriate changes here. The new settings apply for all users and take effect immediately.

To change assay identifier, select which identifier to change and click *Change* button. Make appropriate changes. The delimiter character can be altered in a similar manner. Examples of the new naming will appear in the right lower part of the window.



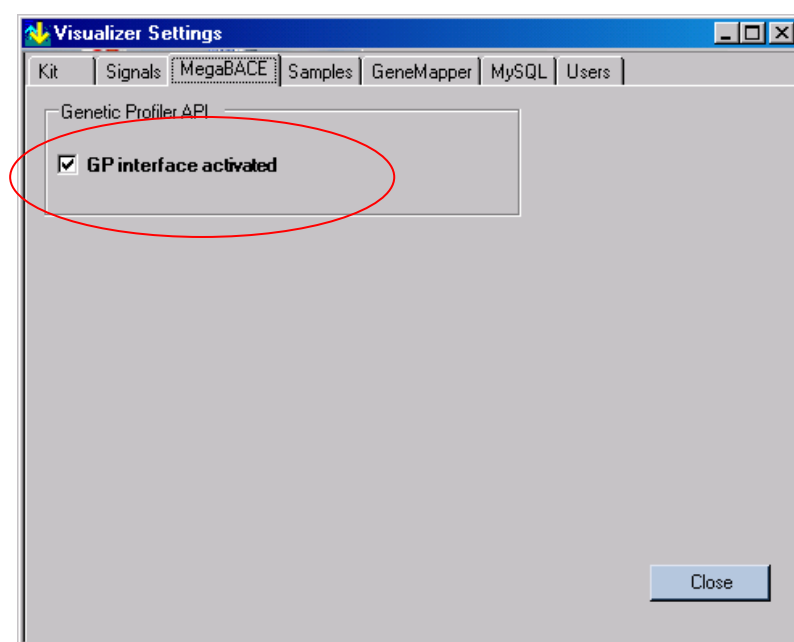
Tip: If you have implemented a solution where you automatically generate samples sheets from your LIM system then these settings can be used to minimize work with integrating your information systems.

4.4 Setting MegaBACE Genetic Profiler

Tools -> Settings -> MegaBACE

This setting is applicable for those who use GE-Healthcare's Genetic Profiler software to size their ChromoQuant data. Genetic Profiler must be installed on the same computer as Visualizer. Genetic Profiler must be closed when running Visualizer.

The Genetic Profiler interface (also known as API) is by default not activated. Check the checkbox if you intend to import ChromoQuant data from a MegaBACE system. This change does not take effect until Visualizer is restarted. All users are affected by the change. Once activated, Visualizer handles both ABI and MegaBACE data.



Restart the Visualizer software after this change.

Note: Genetic Profiler must be installed on the same computer as Visualizer. Unless this is the case it is not possible to import data from a MegaBACE system into Visualizer.

Note: If the GP interface is activated on a system where Genetic Profiler is not installed then Visualizer will issue error messages. You must install GP in order to avoid this.

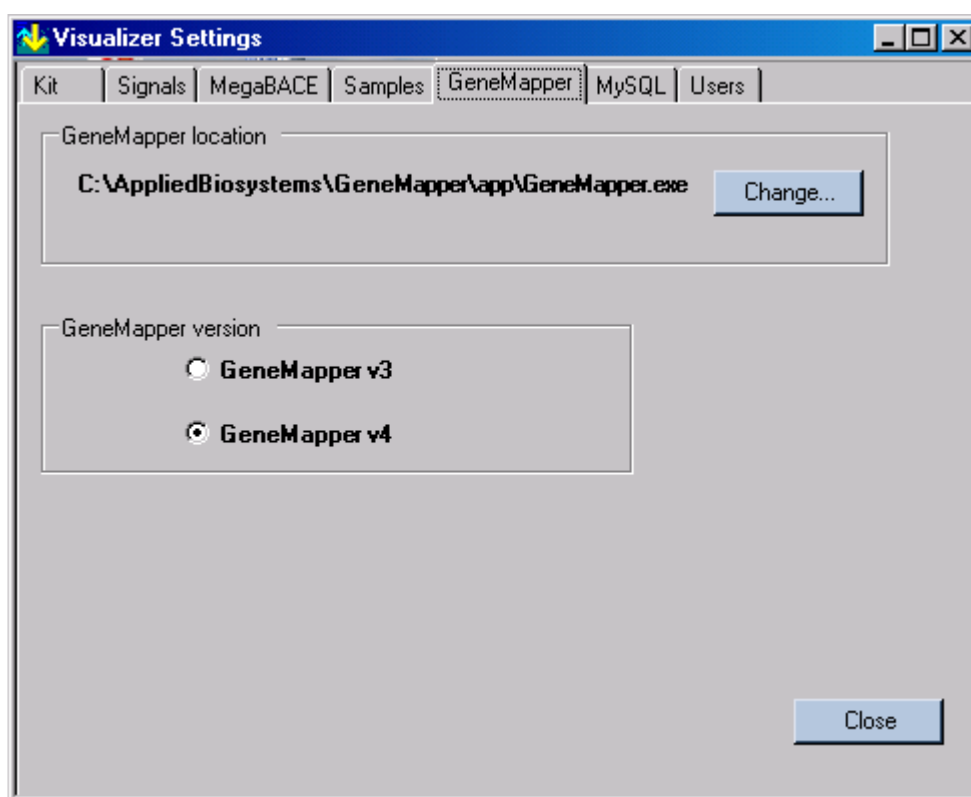
4.5 Setting ABI GeneMapper properties

Tools -> Settings -> GeneMapper

This setting is applicable for those who use ABI's GeneMapper software to size their ChromoQuant data.

Visualizer need the path to GeneMapper.exe when installing Visualizer the path is set to the default value where ABI normally installs GeneMapper. However, if you have a non-default GeneMapper installation or if you must connect to GeneMapper over a network connection (you must map GeneMapper on a logical disk) then this setting can be used. The change is effectuated after restart of Visualizer and applies for all users.

Visualizer supports GeneMapper versions 3 and 4. The versions differ somewhat and therefore you must specify which version of GeneMapper that you are using.



Now, continue to section 7 for Importing data

6.6 *Setting ABI GeneScan properties*

As of Visualizer v2.2, there is no need to provide any settings for GeneScan users. Proceed directly to the Import section

4.6 Setting MySQL properties

Tools -> Settings -> MySQL

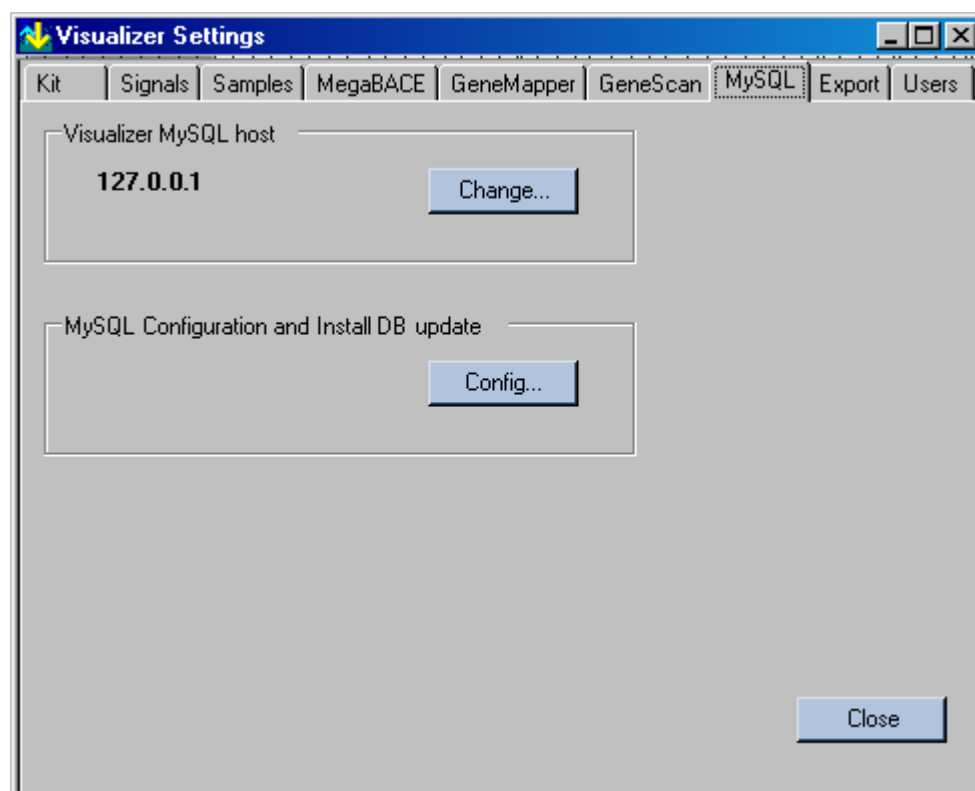
MySQL is the database server that manages Visualizer's database. The database contains ChromoQuant marker information, genotypes and all information about the samples you have imported. The database is vital for Visualizer's functionality and MySQL must be correctly configured otherwise Visualizer will not function properly.

These settings require good understanding of you system installation. If you feel uncertain about these settings then we advice you contact you local system support or CyberGene's support chromoquant@cybergene.se. Normally the MySQL settings never need to be changed.

If MySQL for some reason is installed on a different server host then the IP address setting must be changed. This change can be effectuated here. Note that user privileges and other applicable security policies must allow this connection to be established.

The *Config* button opens up the configuration/installation dialogue that opened first time Visualizer was used. Here you can reinstall MySQL components and/or change settings. For details about configuring MySQL please refer to Appendix 3 or the Installation guide.

Note: Reinstalling Visualizer's database can lead to irrevocably loss of your imported ChromoQuant samples.



4.7 Setting user properties

Tools -> Settings -> Users

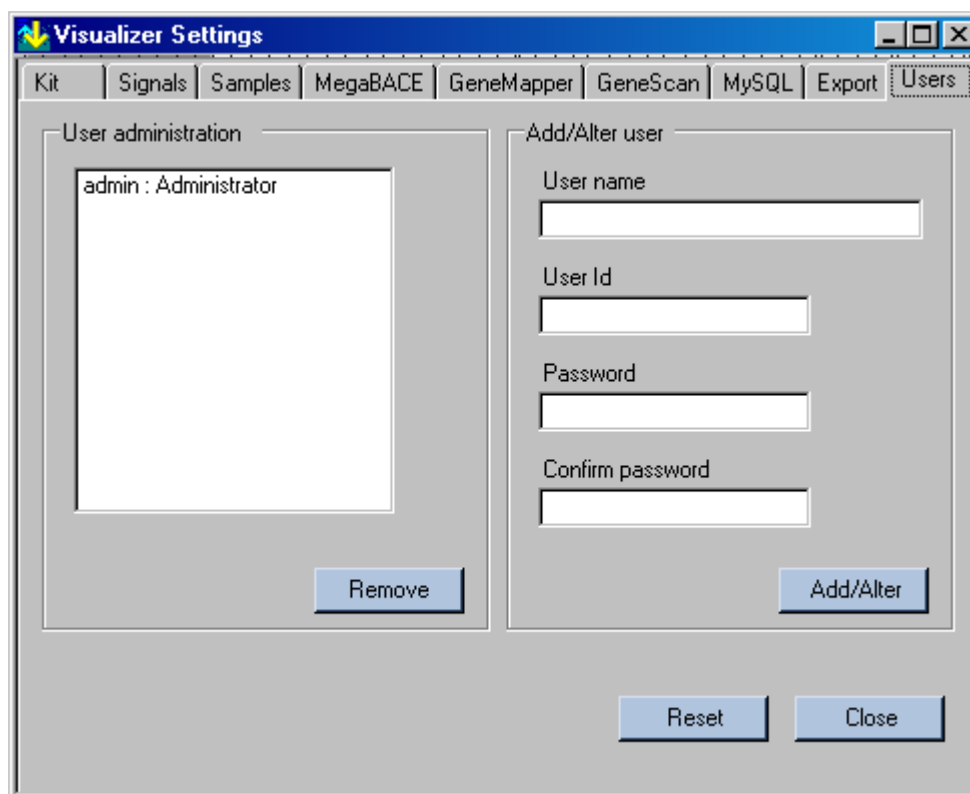
If you login as *Administrator* then you have rights to add/change/remove user profiles.

All user profiles are listed in the listbox in the *User administration* section. Details of a selected profile are displayed in the *Add/Remove user* section.

If a selected profile is to be permanently removed then click the *Remove* button. Changes are edited in the *Add/Remove user* section, password must always be given, click *Add/Alter* to effectuate.

A new user can be entered by first removing any previous selection by clicking *Reset* and thereafter enter user profile data. You must click *Add/Alter* to insert the new user profile. The profile will appear in the listing afterwards.

Users that are not *Administrator* can change their password.



Note: If you forget your *Administrator* password then contact chromomoquant@cybergene.se for instructions how to circumvent this problem.

4.8 Setting export properties

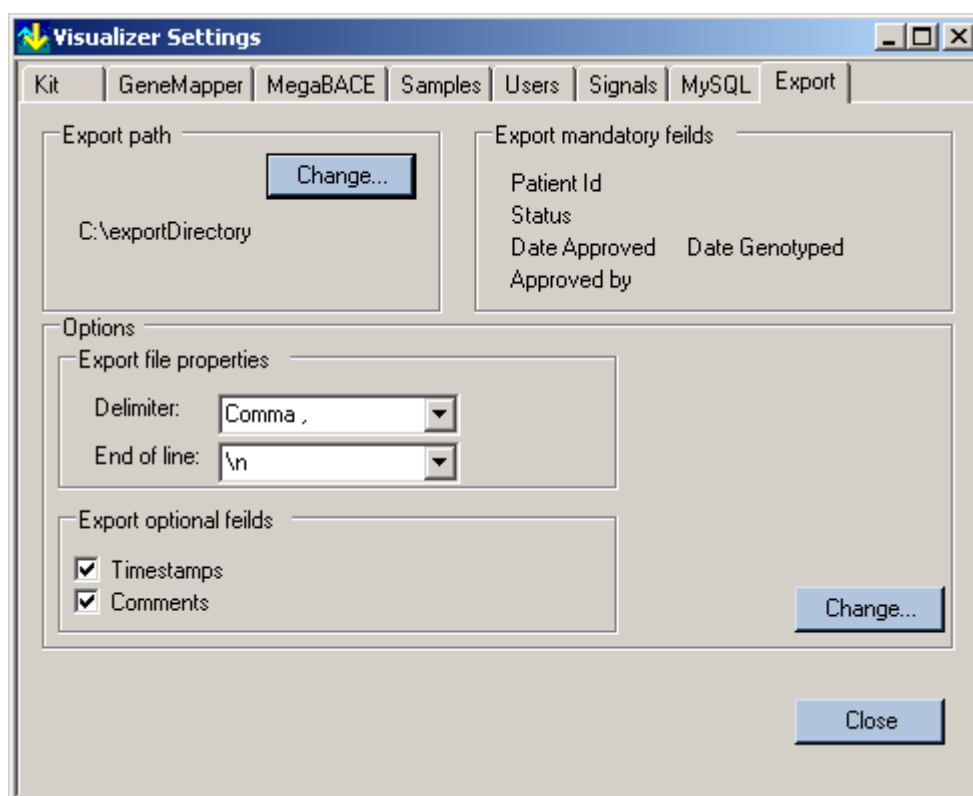
Tools -> Settings -> Export

This function is used by those who want to export the results into some other patient handling system. The export function creates delimited text files with sample data.

Export path determines where Visualizer will save export files. If no directory has been specified then the files will be saved directly on your C drive (C:\). Note that it is possible to set the export path to a network drive. Contact your local IT support to determine a suitable export directory.

The “Export mandatory fields” cannot be altered. These fields will always be exported.

The “Delimiter” combo box allows you to select delimiter from predefined values. You can choose delimiter as *comma*, *semi colon* or *colon*. The “End of line” setting determines how each line in the export file ends. Either it is a Unix style “\n” or a standard ASCII CrLf.



The check boxes in the “Export optional fields” sections allow you to include or omit non mandatory fields in your export files.

Note: Changes to export options are not effectuated until you have clicked the *Change* button.

5 Importing data from different analysis software

Visualizer can import sized ChromoQuant data from all ABI and MegaBACE sequencers . In this section we describe the process of importing sized data into Visualizer. To get precise instructions for how to handle sequencers and their respective software please refer to applicable user documentation.

The analyzed data must first be converted to a format that Visualizer can handle. This is done automatically but you must give the commands to the Visualizer software.

When the data has been converted then the software will check the naming standard. If the names are not properly set, the names can be altered. When everything is OK, the data is imported into the software and the database. Depending upon what analysis instrument you are using in your laboratory you must choose the proper section below:

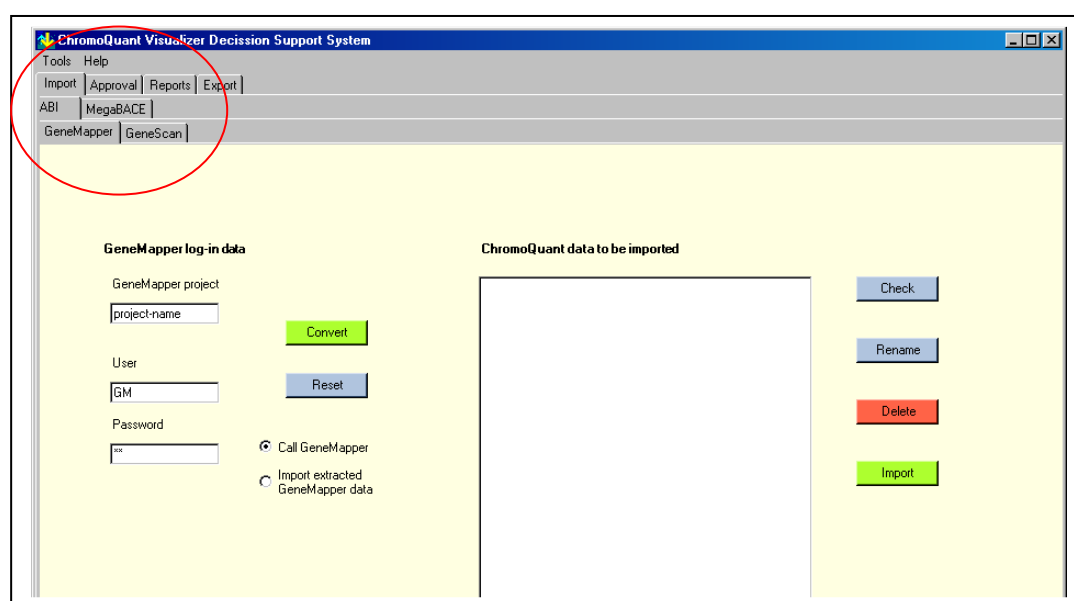
5.1 ABI GeneMapper versions 3 and 4

For configuring Visualizer after your GeneMapper version please refer to the settings section. The data import varies depending on if Visualizer is installed on the same computer as GeneMapper or not. Follow the procedure applicable to your installation.

5.1.1 Procedure when GeneMapper and Visualizer are installed on the same computer:

Start GeneMapper and create a new GeneMapper project by importing the sample files created in your run. The sample names are set in the sample sheet. In order to avoid extra work we advice to use naming conventions as described in section 4. Assign correct size standard to the files and analyze them in GeneMapper. Samples that fail to be sized should be removed since these need to be re-run under all circumstances. When the project has been successfully sized then close it and exit GeneMapper.

Start Visualizer and select the *Import -> ABI -> GeneMapper* tabs.



Enter the exact name of the project, containing your sized ChromoQuant data. In the User/Password fields, enter your GeneMapper login details.

Call GeneMapper is checked in the radio-button. This indicates that Visualizer will call GeneMapper and retrieve all ChromoQuant samples from the project you specified. The process to call GeneMapper can in some circumstances take some time, depending on your project's size it can take up to several minutes to make the call. Please wait until the data appears in the window to the right and do not attempt to close any GeneMapper windows that might open up during the import.

Note: In the settings (see section 6.5) you have to specify which GeneMapper version that you are using and its location. Default location is on the same computer as Visualizer. GeneMapper can be located to any ne2rk-connected computer.

Continue to section 7.2.

5.1.2 Procedure when GeneMapper resides on a different computer than Visualizer:

Use the GeneMapper retriever utility software (see the Visualizer GeneMapper Retriever manual for instructions).

Open the GeneMapper retriever software.

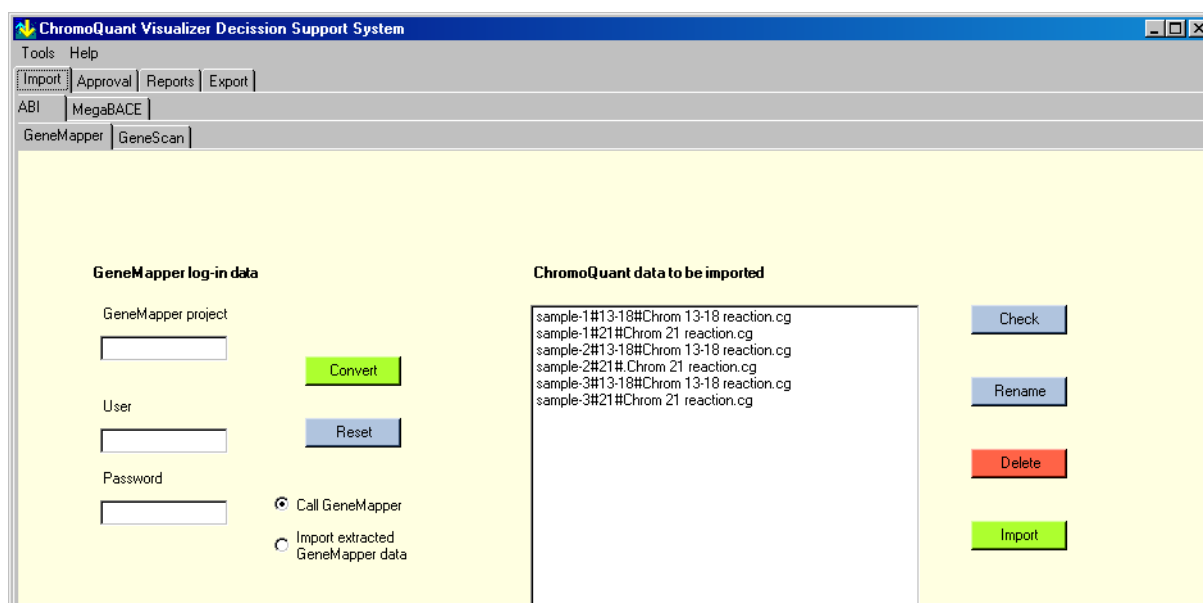
When the *import extracted data* radio button is checked then a file selection dialogue box will appear. Navigate to the folder where you placed your extracted data from GeneMapper Retriever. Export your GeneMapper project to a folder that can be accessed by Visualizer. Note, if there is no network connection you have to move the export directory to a USB-memory.

In Visualizer, check the other radio button “Import extracted GeneMapper data” to indicate that GeneMapper data already has been extracted into a separate folder.

Thereafter, click the *Convert* button and the import from GeneMapper will start. First you will be prompted to identify the folder containing the previously exported GeneMapper project. After importing the project, your ChromoQuant sample names will appear in the list box to the right containing samples to be imported.

The imported ChromoQuant data is now displayed in the list box. Here you can check the validity of the file names, that is, that the file name adheres to the naming convention and that there already is no duplicate name in Visualizer's database. If the file name needs to be corrected then a file dialogue box is opened where you can do this.

Tip: If your sample sheet naming convention is not the same as Visualizer's then just convert the data and rename your samples in Visualizer.



If you want to re-name or delete any of the ChromoQuant files imported from your GeneMapper project then select one or multiple samples (press **CTRL** and click on all samples you want to select, click on the **RESET** button to remove any selection). Click the **RENAME** or **DELETE** button.

Continue to section 7.2.

5.2 ABI GeneScan

Create a new GeneScan project and insert the sample files created in your run. The sample names are set in the sample sheet and we advice to use naming conventions as described in the preceding section.

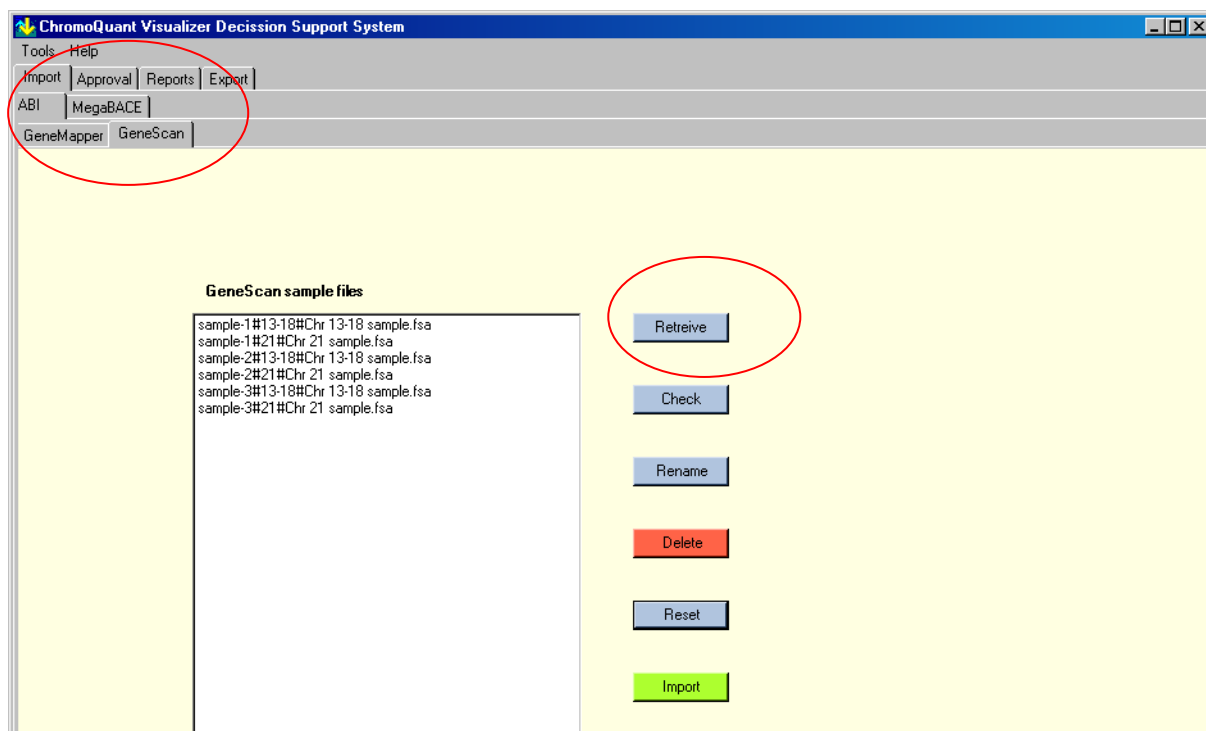
Assign correct size standard to the files and analyze them in GeneScan. Samples that fail to be sized should be removed since these need to be rerun under all circumstances. When the project has been successfully sized then close it and exit GeneScan.

Start *Visualizer* and select the *Import -> ABI -> GeneScan* tab.

Click the *Retrieve* button in order to open a file selection window. Navigate to the folder where you saved your GeneScan project “Run folder” and select the folder. Click *Ok*.

The imported ChromoQuant data is now displayed in the list box. Here you can check the validity of the file names, that is, that the file name adheres to the naming convention and that there already is no duplicate name in Visualizer’s database. If the file name needs to be corrected then a file dialogue box is opened where you can do this.

If you want to rename or delete any of the ChromoQuant files imported from your GeneMapper project then select one or multiple samples (press *Ctrl* and click on all samples you want to select, click on the *Reset* button to remove any selection). Click the *Rename* or *Delete* button.



Tip: If your sample sheet naming convention is not the same as Visualizer's then mark a sample and rename choosing the "Rename" button or simply choose "Check".

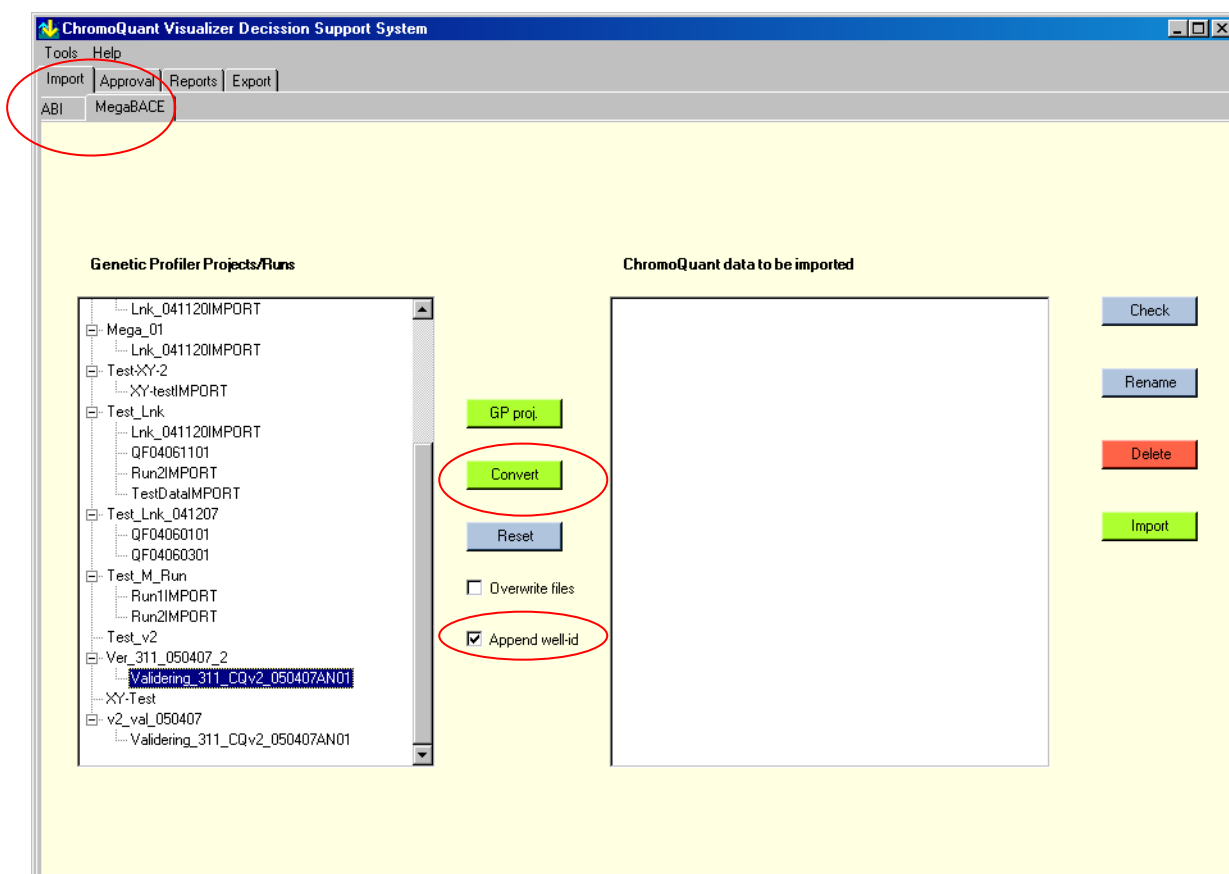
5.3 GE-Healthcare Genetic Profiler

Note: Genetic Profiler must be installed on the same computer where Visualizer is installed. In order to import Genetic Profiler data you must activate the GP interface in *Settings* (see section 6.5).

Create a new Genetic Profiler (GP) project containing the sample files created in your run. The sample names are set in the sample sheet and we advice to use naming conventions as described in the preceding section.

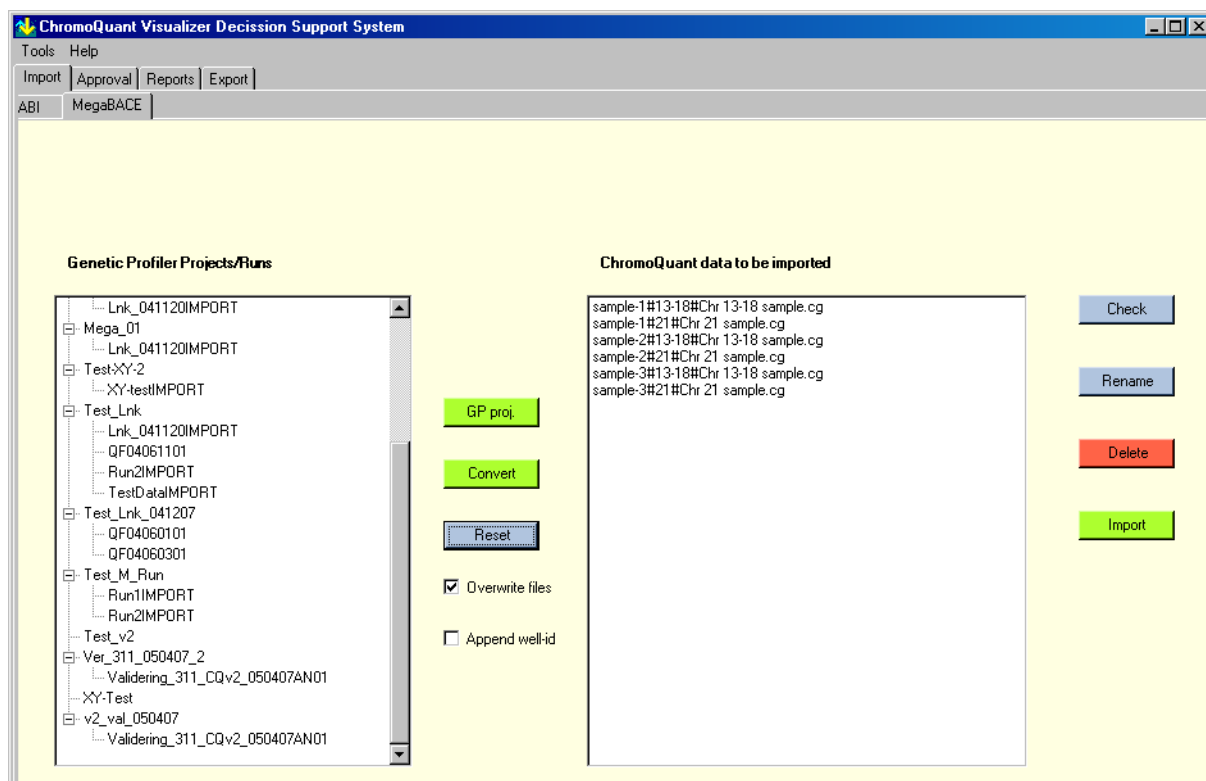
Assign correct size standard to the files and analyze them in Genetic Profiler. Samples that fail to be sized should be removed since these need to be rerun under all circumstances. When the project has been successfully sized then close it and exit Genetic Profiler.

Start *Visualizer* and select the *Import -> MegaBACE* tab. Click the *GP proj.* button to display a tree view of your GP projects. Each project can be expanded in order to display the runs associated with the project. Select the run of your project containing the ChromoQuant data that you want to analyze and click the *Convert* button.



The two checkboxes *Overwrite files* and *Append well-id* functions as follows; *Overwrite files* allows sample files with duplicate name to be overwritten. This, however, indicates a poor naming standard and we caution for the consequences of using this option. *Append well-id* will append the MegaBACE well-id at the end of the sample name given in the sample sheet. This option can make it simpler to identify particular samples.

Click *Convert* to import the project/run sample data into Visualizer. The imported ChromoQuant samples will appear in the rightmost list box.



The process to call Genetic Profiler and to extract the sample data from the selected project/run, can in some circumstances take some time. Depending on your project size it may take up to a minute to complete the call extraction of data. Please wait until the files appear in the right field of the screen.

To rename or delete any of the ChromoQuant files imported from your GeneMapper project select one or multiple samples (press *Cntrl* and click on all samples you want to select, click on the *Reset* button to remove any selection). Click the *Rename* or *Delete* button.

5.4 Importing the data

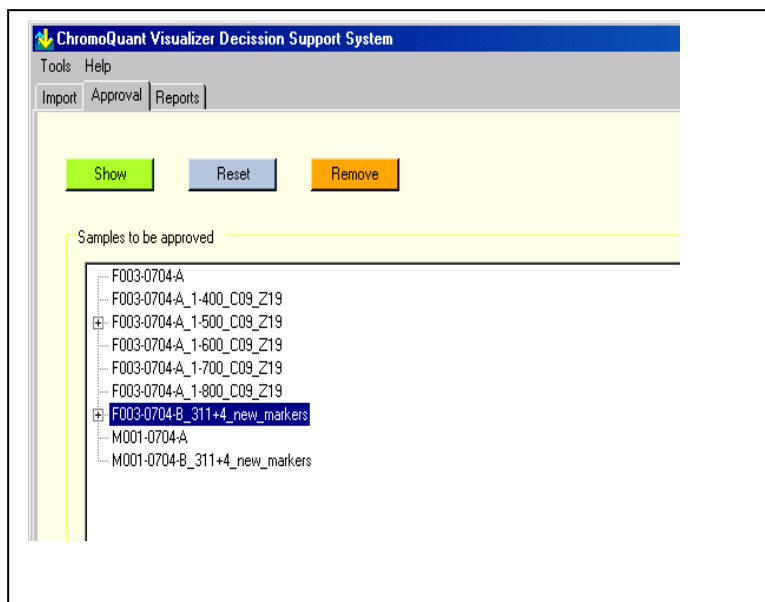
When you are satisfied that your sample names are correct then simply click the *Import* button in order to import and automatically genotype your ChromoQuant samples. A message box will appear informing about which samples that was successfully imported and genotyped. All imported samples are removed from the list of converted samples.

A message will confirm that all your results have been imported properly.

6 Approving samples

The approval function allows you to approve/reject Visualizer's genotyping interpretations. Start by selecting the

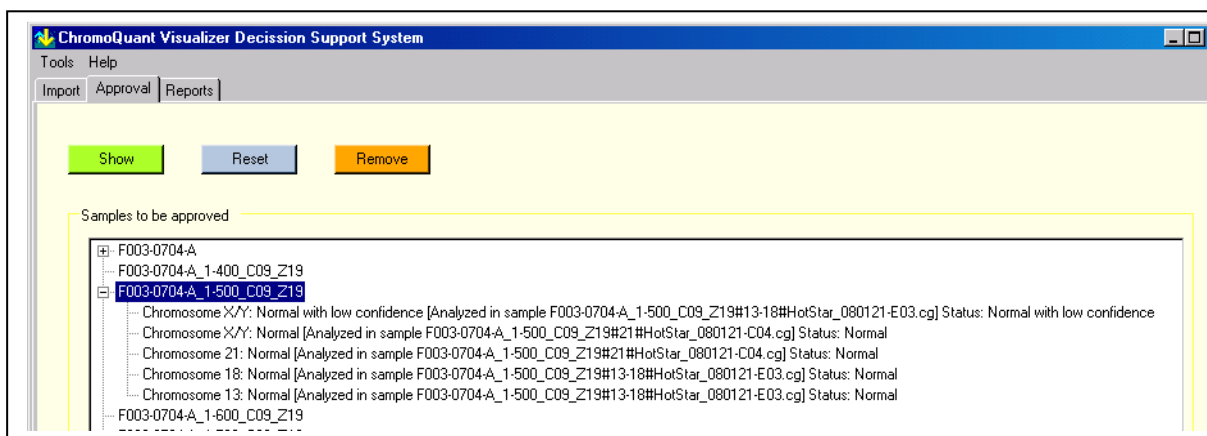
1. *Approval* tab and click
2. *Show* to list all genotyped ChromoQuant samples



The *Reset* button clears previous selections. All genotyped samples appear in the list box. The listing is a tree structure, containing the genotypes for each sample's chromosomes, that can be opened up by selecting one or several samples. This allows you to quickly glance through your genotyped samples.

The *Remove* button removes a marked sample, and all of its associated chromosomes, from the list. Note that removing a sample is irrevocable and should only be used for samples that have been incorrectly imported or when the sample cannot be correctly analyzed.

In order to view details for a chromosome you simply select it by marking it in the list. This action opens up a separate window form containing detailed information about markers and fragment peaks.

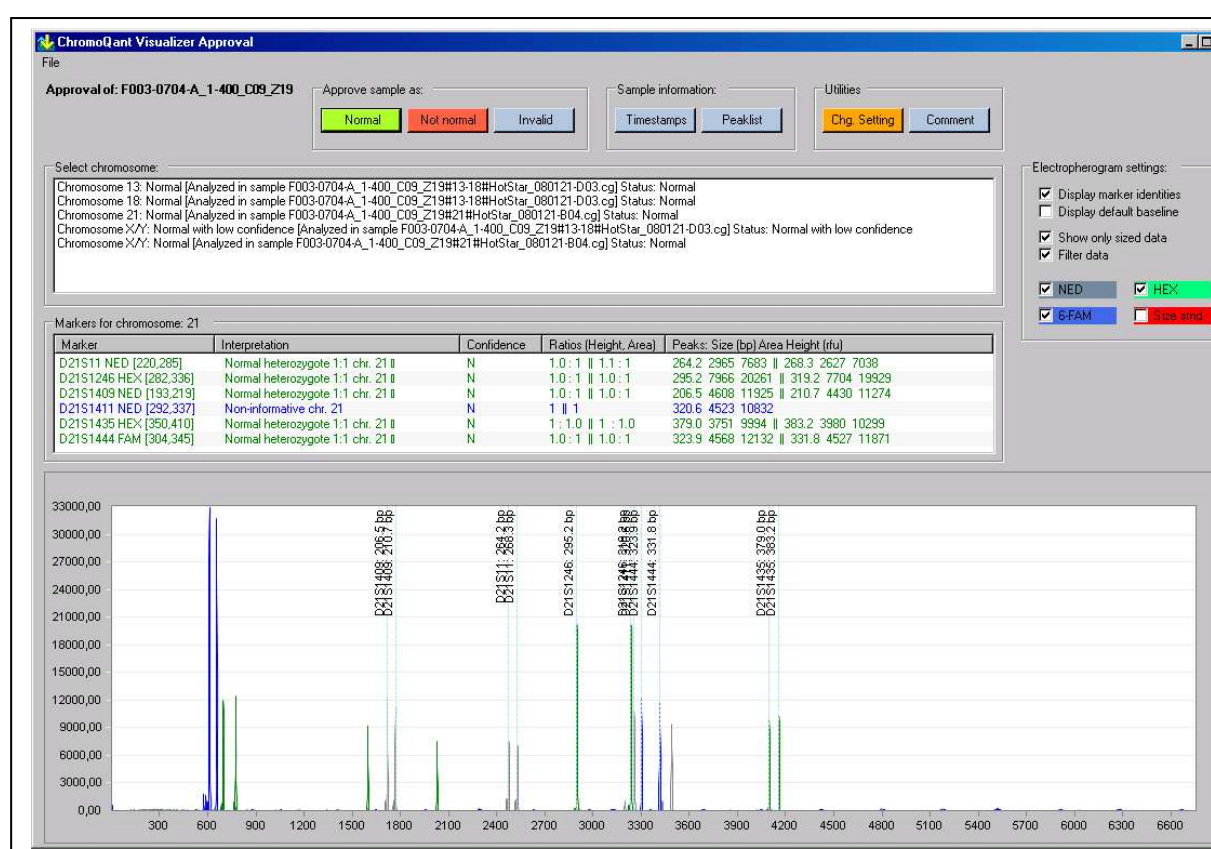


Note: Each chromosome has an interpretation, such as “*Chromosome 21: Normal...*”. The interpretation is based on the genotypes assigned to the chromosome’s markers. However, the sex chromosomes will be presented as “*Normal with low confidence...*” for normal male samples.

Note: Homozygote genotype can be informative when sex chromosome markers are interpreted. For these markers such genotypes will be colour coded differently in order to correctly communicate the interpretation.

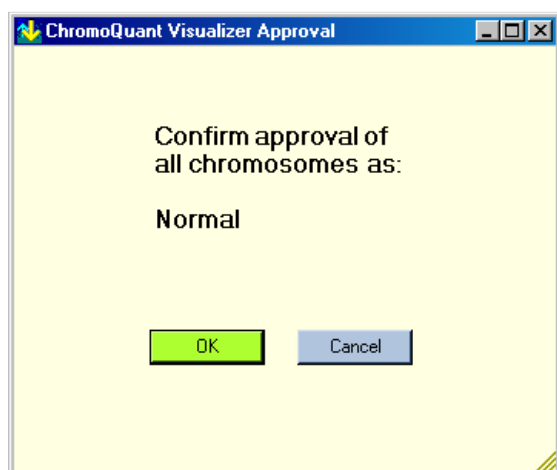
Each marker, associated with selected chromosome, is presented with its colour-coded interpretation. The marker’s fragment peaks (size, height and area) and their calculated quantitative ratios are presented as well.

All chromosomes are displayed in the top right listbox, selecting any one of these re-opens the windows form with the newly selected chromosome’s markers.



Visualizer interprets each marker based on the associated peaks height/area proportions. The resulting interpretation is presented colour coded, to ease usability. Where **green** indicates normal (heterozygote genotype with peak proportion 1:1). **Blue** indicates a non-informative marker (homozygote genotype resulting in one peak wherefrom no quantitative information can be derived). **Red** indicates an aneuploidy (heterozygote genotype with peak proportion 1:2 or 1:1:1). Finally, **orange** indicate a risk genotype where the interpretation either depends on other marker’s interpretation or when the marker’s peak pattern cannot unambiguously be interpreted as either normal or as an aneuploidy.

There are five buttons named *Normal*, *Not normal*, *Invalid*, *Peaklist* and *Timestamps*. The first three buttons allows you to approve the sample and change its status from *genotyped* to *normal*, *not normal* or *invalid*. Approving the sample changes the status for all chromosomes. The approval must be confirmed in order to be effectuated.



Clicking the *Cancel* button leaves the sample's status unchanged and still not approved in the database. Clicking *OK* updates the sample's status and closes the approval window. The sample is then locked for any change.

Clicking the *Peaklist* button will open a window containing all sized peaks for the sample.

ChromoQuant Visualizer Peaklist									
Sample name	Dye	Size (bp)	Height (rfu)	Area	Height ratio	Area ratio	Stutter	Low peak	Overflow
Training-normal_female#13-18#311.cg	RDX	400	2374	1957	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	425	2231	1830	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	450	2241	1891	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	475	2440	2183	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	500	2422	2934	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	525	2419	2543	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	550	2469	2657	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	28,1969	58817	186080	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	28,7272	58614	302766	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	30,1854	61897	170940	0	0	Normal	Normal	Overflow sl...
Training-normal_female#21#311.cg	6-FAM	35,8856	4781	5082	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	37,0787	3455	8284	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	40,3928	380	0	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	44,5023	10078	6137	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	45,4303	6507	6488	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	46,6233	4229	3810	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	47,4187	4806	3802	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	48,4792	2958	4903	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	49,1421	4474	3411	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	49,6723	4331	3667	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	52,5887	851	0	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	53,5167	463	0	0	0	Stutter	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	107,111	141	51	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	108,87	125	42	0	0	Stutter	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	109,998	5940	2593	1	1	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	114,236	6258	2674	1,05354	1,03124	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	319,904	179	57	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	323,873	5222	2790	1,1589	1,10714	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	331,87	4506	2520	1	1	Normal	Normal	Normal
Training-normal_female#21#311.cg	HEX	28,3295	27162	9262	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	HEX	29,7877	28983	11508	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	HEX	30,5921	20067	9575	0	0	Stutter	Normal	Normal

Sizing information is given for each peak. A colour coding indicates peak status. Valid peaks are listed in green color. Blue color for peaks that has been filtered out, peaks below the baseline or peaks that are below 20% of the highest peak in a marker region. Stutter peaks are also filtered and indicated in blue colour. Those peaks that are within a marker region and where the height exceeds the overflow threshold are indicated in orange colour – see *Signal settings*.

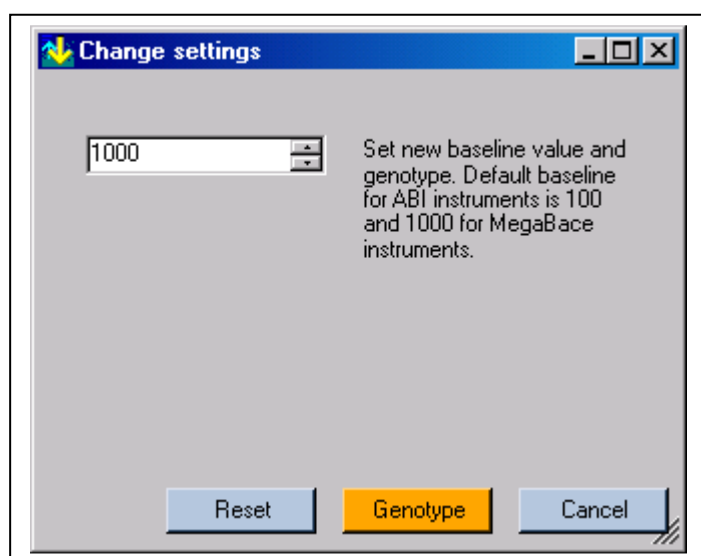
A peak that is within a marker region and not filtered out, i.e. a valid (green) marker peak, has its QF-PCR respective ratio included in the peaklist.

The *Timestamp* window contains a listing of all timestamps belonging to the sample.

ChromoQuant Visualizer Timestamps			
Sample name	User	Timestamp	Action
Training-normal_female#13-18#311.cg	Administrator	2007-12-23 11:10:08	Sample analyzed and genotyped
Training-normal_female#13-18#311.cg	Administrator	2007-12-23 11:10:08	Registered and associated with Training-normal_female
Training-normal_female#21#311.cg	Administrator	2007-12-23 11:10:08	Sample analyzed and genotyped
Training-normal_female#21#311.cg	Administrator	2007-12-23 11:10:08	Registered and associated with Training-normal_female
Training-normal_female#*YE#*YE.cg	Administrator	2007-12-23 11:10:09	Sample analyzed and genotyped
Training-normal_female#*YE#*YE.cg	Administrator	2007-12-23 11:10:09	Registered and associated with Training-normal_female

This function is to give a full traceability of actions performed in the system. Each timestamp contains information about which user that performed the timestamp action and a descriptive text. Timestamps are part of the information that can be exported. For further details please refer to the section about Exporting data.

The *Change Settings* will allow you to alter the base line used for genotyping. This might be useful, for instance if you have a high base-line that disturbs the interpretation, or alternatively if you data is below the default base-line and therefore has not been analysed.



The 'Change settings' dialog box features a title bar with a green arrow icon and standard window controls. Inside, there is a text input field containing the value '1000'. To the right of the input field, a text label reads: 'Set new baseline value and genotype. Default baseline for ABI instruments is 100 and 1000 for MegaBace instruments.' At the bottom of the dialog, there are three buttons: 'Reset' (light blue), 'Genotype' (orange), and 'Cancel' (light blue).

After changing the base line settings the sample is Genotyped with the new setting. The change will be traceable in the Timestamps. You can also add your own comment by using the *Comment* button.

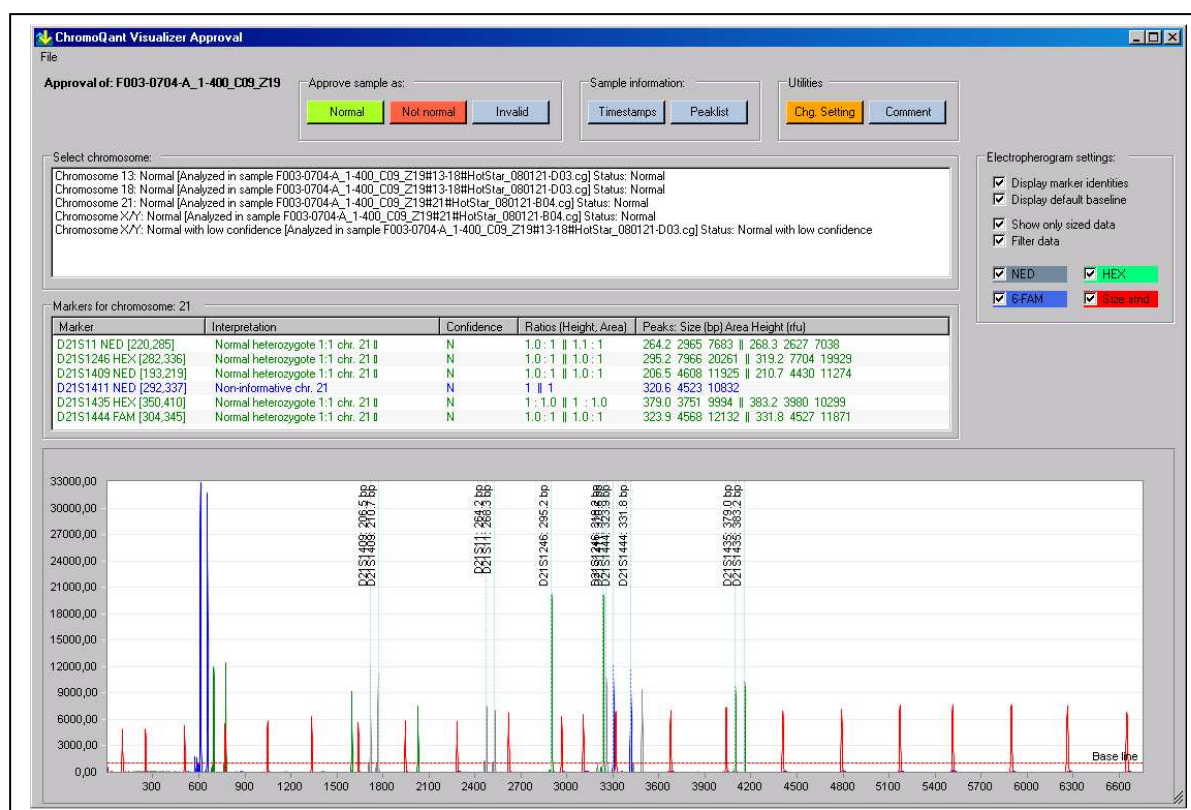
In the main Trace data window there are four checkboxes that control the appearance of the electropherogram. *Display marker identities* controls if marker information is to be included in the electropherogram or not. *Display default baseline* is useful when the standard settings have been changed.

Show only sized data shows only the part of the electropherogram in-between, and including, the first and last size standard peak. If this checkbox is unchecked the entire electropherogram is traced including all initial primer peaks. The electropherogram is always rescaled in order to optimize readability. Finally, *Filter data* removes artefact signals improving readability of the electropherogram.

Tip: Sometimes readability can be improved by forcing rescaling of the electropherogram. Just uncheck and recheck any of the checkboxes. This action will initiate rescaling.

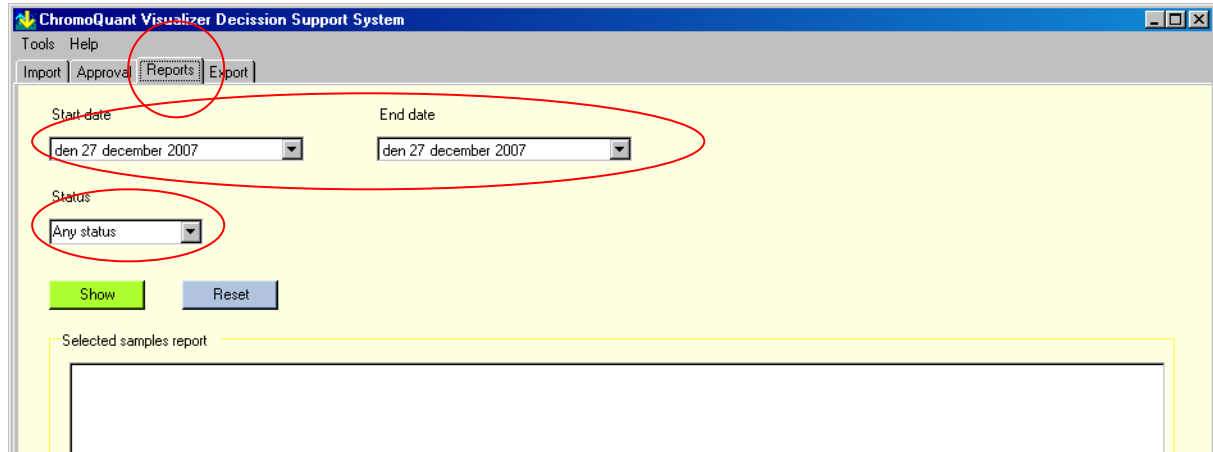
The confidence value given for each marker interpretation can be **N** (normal), **U** (uncertain) or **L** (low). *Normal* confidence is assigned to interpretation where peak separation is less than 22bp for consecutive peaks and where peak ratios (normalized height and area) fall in either of the intervals [1,1.4] or [1.8,2.2].

When peak separation exceed 22bp then both intervals are increased by 0.1 to [1,1.5] and [1.7,2.3]. When both height and area ratios fall within these intervals then the assigned confidence is *uncertain*. The assigned confidence is *low* when either one of height/area ratios fail to fall within stipulated intervals.



7 Reports

The report function allows you to select ChromoQuant samples based on the date when the sample was imported into Visualizer and optionally on the samples status. Start by selecting the *Reports* tab.



You must always specify a time interval for your report. If Start and End date are the same then the time interval span over one day.

Tip: Visualizer's database contain training samples that have import date January 06 2008. To view these samples you have to select start date January 06 2008 and *Any Status*.

Tip: Visualizer makes use of the calendar function in Windows to determine the time interval. Sometimes a samples "timestamp" can be on the boundary of a time interval. In order to ensure that you retrieve all intended samples *you can increase the time interval with one day*.

Note: Visualizer makes use of the Windows clock. If the clock and/or time zone is changed then timestamps prior to the change will be "out of sync". Changing the clock can also cause the relative order between timestamps to be disrupted.

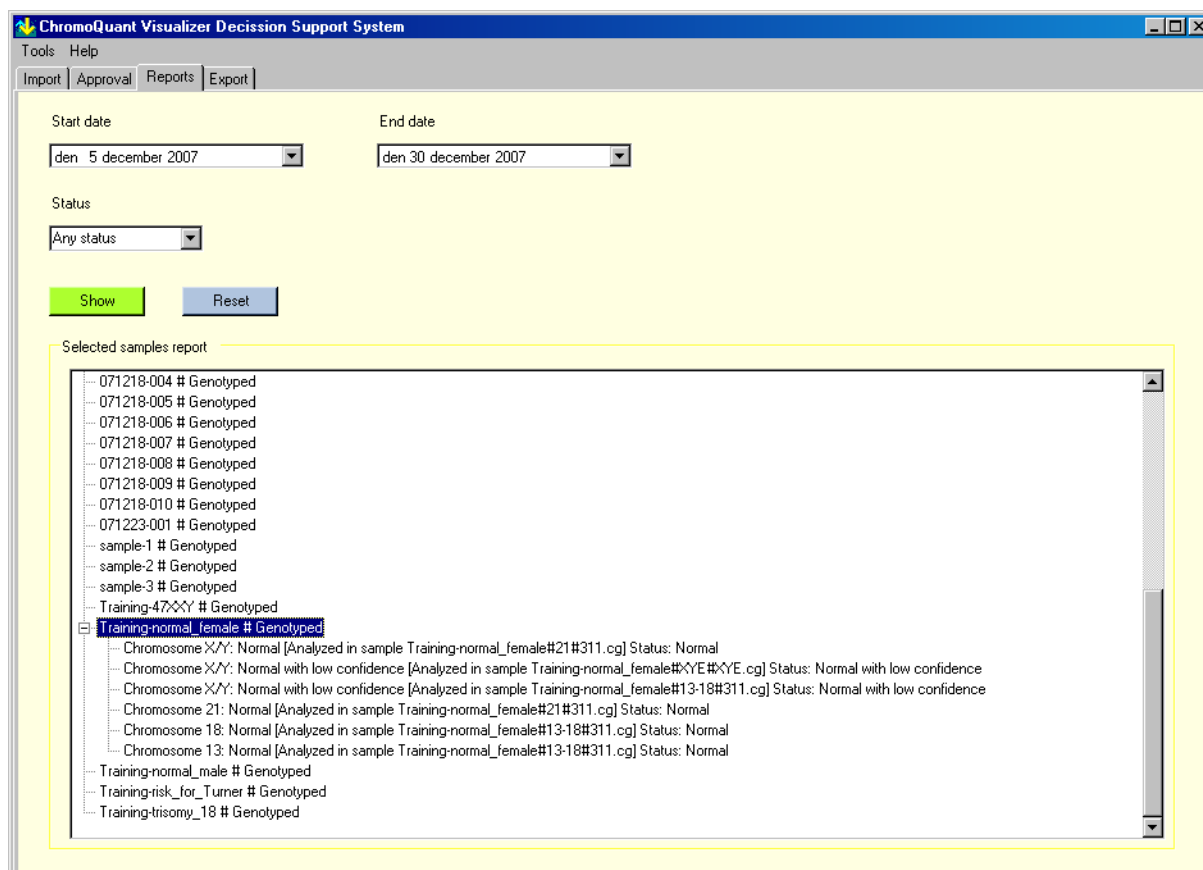
After selecting a time period you can optionally specify the samples' status. A sample can have the following status codes:

- Genotyped
- Approved
- Exported

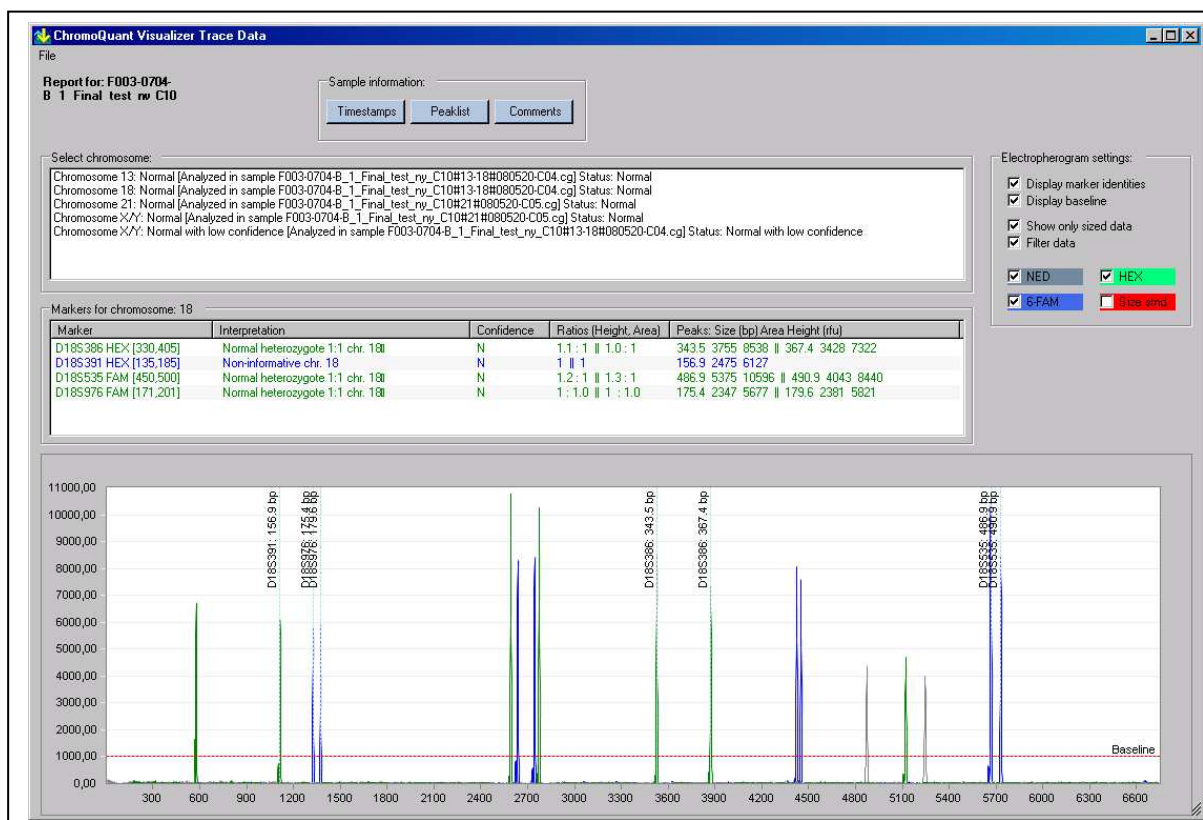
Generate the report by clicking the *Show* button. The *Reset* button clears previous selections.

The selected samples together with their status appears in the list box. The listing is a tree structure, containing the genotypes for each sample's chromosomes, that can be opened up by selecting one or several samples. This allows you to quickly glance through your selected samples.

Please observe that in this view nothing can be changed or added. If the sample has been approved then it is locked for all future and stored in the database. The report view can only be used for studying the data and **Printing** the results.



In order to view details for a chromosome you simply select it by marking the chromosome text. This action opens up a separate window form containing detailed information about markers and fragment peaks.



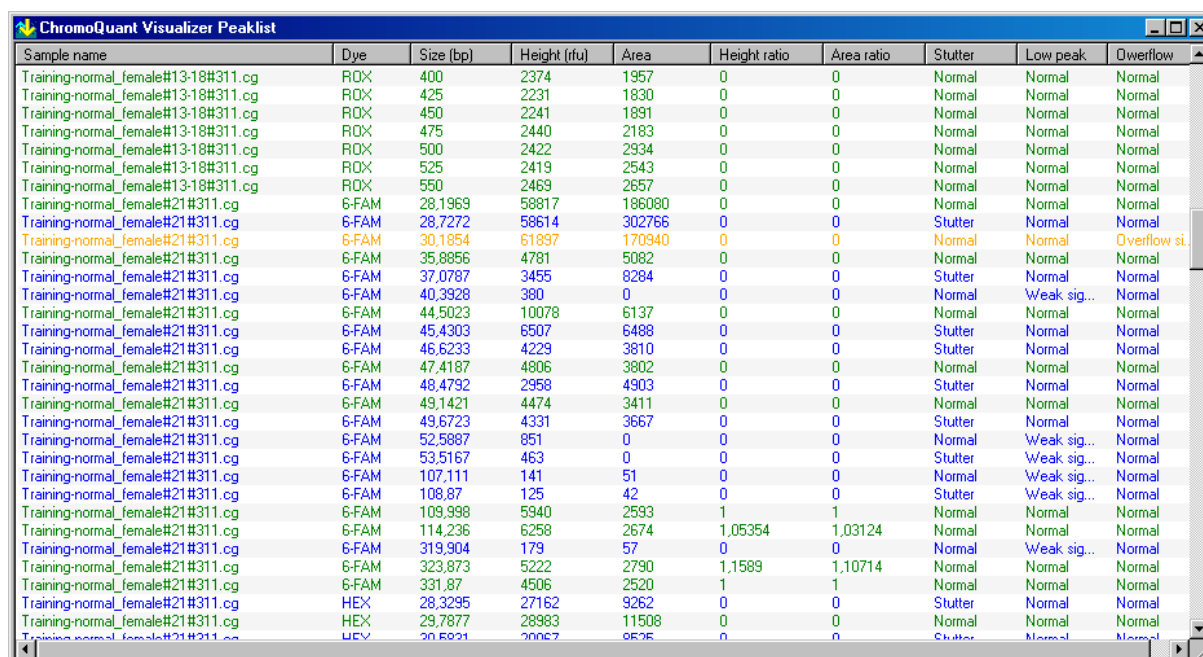
Visualizer interprets each marker based on the associated peaks height/area proportions. The resulting interpretation is presented colour coded, to ease usability. Where **green** indicates normal (heterozygote genotype with peak proportion 1:1). **Blue** indicates a non-informative marker (homozygote genotype resulting in one peak wherefrom no quantitative information can be derived). **Red** indicates an aneuploidy (heterozygote genotype with peak proportion 1:2 or 1:1:1). Finally, **orange** indicate a risk genotype where the interpretation either depends on other marker's interpretation or when the marker's peak pattern cannot unambiguously be interpreted as either normal or as an aneuploidy.

Note: Homozygote genotype can be informative when sex chromosome markers are interpreted. For these markers such genotypes will be colour code differently in order to correctly communicate the interpretation.

Each marker, associated with selected chromosome, is presented with its colour-coded interpretation. The marker's fragment peaks (size, height and area) and their calculated quantitative ratios are presented as well.

All chromosomes are displayed in the top listbox, selecting any one of these re-opens the windows form with the newly selected chromosome's markers.

There are three buttons named *Peaklist*, *Timestamps* and *Comments*. Clicking the peaklist button will open a window containing all sized peaks for the sample.



Sample name	Dye	Size (bp)	Height (rfu)	Area	Height ratio	Area ratio	Stutter	Low peak	Overflow
Training-normal_female#13-18#311.cg	RDX	400	2374	1957	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	425	2231	1830	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	450	2241	1891	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	475	2440	2183	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	500	2422	2934	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	525	2419	2543	0	0	Normal	Normal	Normal
Training-normal_female#13-18#311.cg	RDX	550	2469	2657	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	28,1969	58817	186080	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	28,7272	58614	302766	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	30,1854	61897	170940	0	0	Normal	Normal	Overflow si...
Training-normal_female#21#311.cg	6-FAM	35,8856	4781	5082	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	37,0787	3455	8284	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	40,3928	380	0	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	44,5023	10078	6137	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	45,4303	6507	6488	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	46,6233	4229	3810	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	47,4187	4806	3802	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	48,4732	2958	4903	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	49,1421	4474	3411	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	49,6723	4331	3667	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	52,5887	851	0	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	53,5167	463	0	0	0	Stutter	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	107,111	141	51	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	108,87	125	42	0	0	Stutter	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	109,998	5940	2593	1	1	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	114,236	6258	2674	1,05354	1,03124	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	319,904	179	57	0	0	Normal	Weak sig...	Normal
Training-normal_female#21#311.cg	6-FAM	323,873	5222	2790	1,1589	1,10714	Normal	Normal	Normal
Training-normal_female#21#311.cg	6-FAM	331,87	4506	2520	1	1	Normal	Normal	Normal
Training-normal_female#21#311.cg	HEX	28,3295	27162	9262	0	0	Stutter	Normal	Normal
Training-normal_female#21#311.cg	HEX	29,7877	28983	11508	0	0	Normal	Normal	Normal
Training-normal_female#21#311.cg	HEX	30,5921	20067	9535	0	0	Stutter	Normal	Normal

Sizing information is given for each peak. A colour coding indicates peak status. Valid peaks are listed in green color. Blue color for peaks that has been filtered out, peaks below the baseline or peaks that are below 20% of the highest peak in a marker region. Stutter peaks are also filtered and indicated in blue colour. Those peaks that are within a marker region and where the height exceeds the overflow threshold are indicated in orange colour. A peak that is within a marker region and not filtered out, i.e. a valid (green) marker peak, has its QF-PCR respective ratio included in the peaklist.

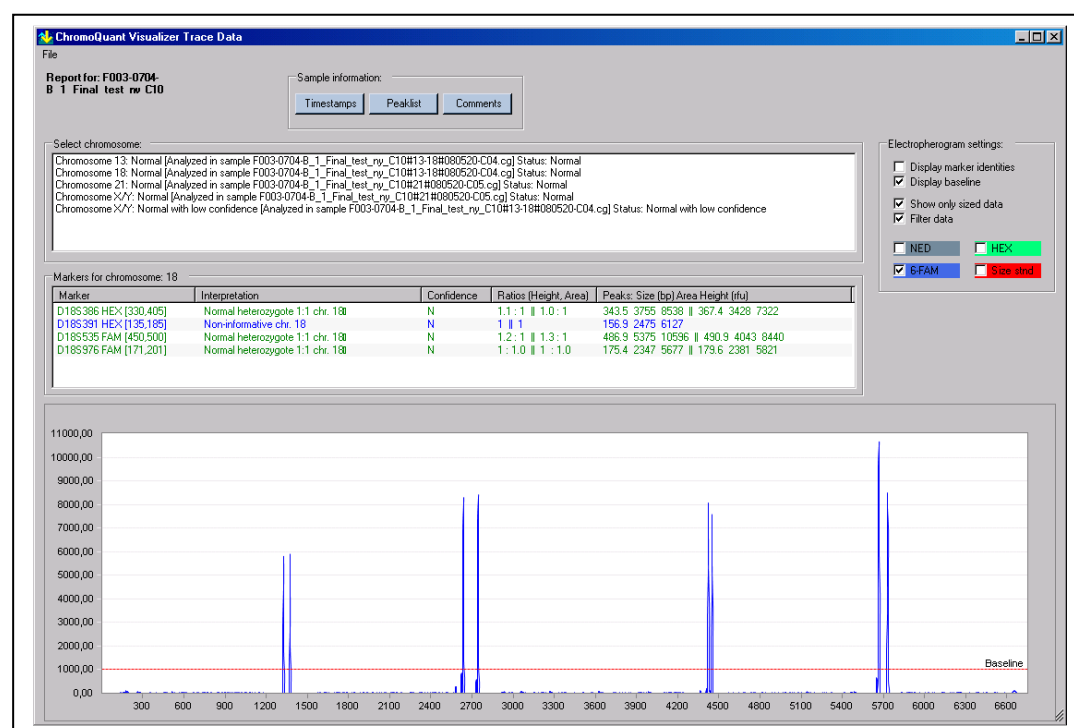
The *Timestamp* window contains a listing of all timestamps belonging to the sample.

ChromoQuant Visualizer Timestamps			
Sample name	User	Timestamp	Action
Training-normal_female#13-18#311.cg	Administrator	2007-12-23 11:10:08	Sample analyzed and genotyped
Training-normal_female#13-18#311.cg	Administrator	2007-12-23 11:10:08	Registered and associated with Training-normal_female
Training-normal_female#21#311.cg	Administrator	2007-12-23 11:10:08	Sample analyzed and genotyped
Training-normal_female#21#311.cg	Administrator	2007-12-23 11:10:08	Registered and associated with Training-normal_female
Training-normal_female#XYE#XYE.cg	Administrator	2007-12-23 11:10:09	Sample analyzed and genotyped
Training-normal_female#XYE#XYE.cg	Administrator	2007-12-23 11:10:09	Registered and associated with Training-normal_female

This function is to give a full traceability of actions performed in the system. Each timestamp contains information about which user that performed the timestamp action and a descriptive text. Timestamps are part of the information that can be exported. For further details please refer to the section about Exporting data.

In the main Trace data window there are four checkboxes that control the appearance of the electropherogram. *Display marker identities* controls if marker information is to be included in the electropherogram or not. *Display baseline* will show the level of the default baseline used for genotyping. *Show only sized data* shows only the part of the electropherogram in-between, and including, the first and last size standard peak. If this checkbox is unchecked the entire electropherogram is traced including all initial primer peaks. The electropherogram is always rescaled in order to optimize readability. Finally, *Filter data* removes artefact signals improving readability of the electropherogram.

You can also choose to look at the data in separate dyes. For instance in the above data, you can take away NED and HEX and just look at the blue data:



Tip: Sometimes readability can be improved by forcing rescaling of the electropherogram. Just uncheck and recheck any of the checkboxes. This action will initiate rescaling.

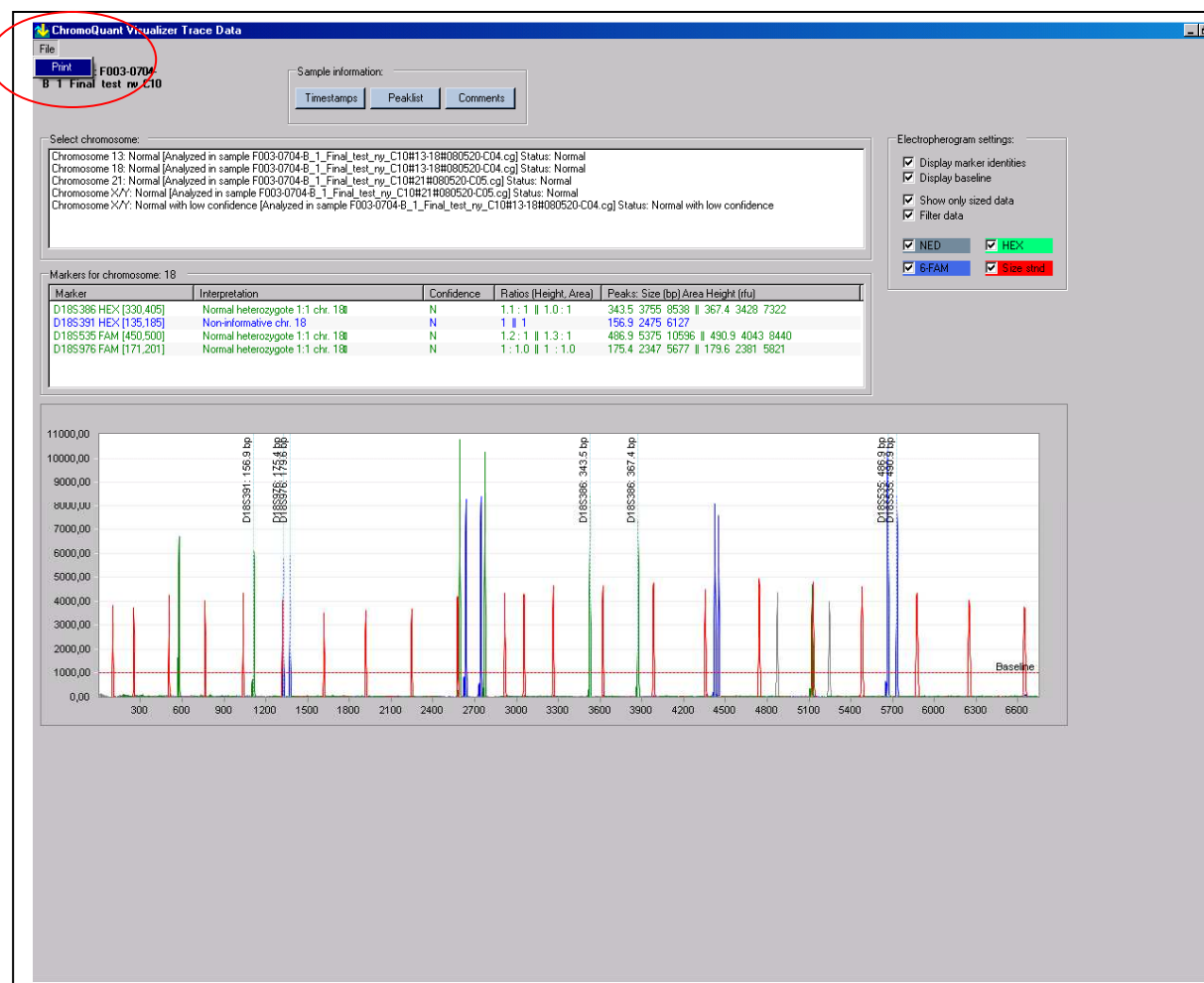
The confidence value given for each marker interpretation can be **N** (normal), **U** (uncertain) or **L** (low). *Normal* confidence is assigned to interpretation where peak separation is less than 22bp for consecutive peaks and where peak ratios (normalized height and area) fall in either of the intervals [1,1.4] or [1.8,2.2].

When peak separation exceed 22bp then both intervals are increased by 0.1 to [1,1.5] and [1.7,2.3]. When both height and area ratios fall within these intervals then the assigned confidence is *uncertain*. The assigned confidence is *low* when either one of height/area ratios fail to fall within stipulated intervals.

8 Printing results

In the Report view, choose *Tools -> Print*

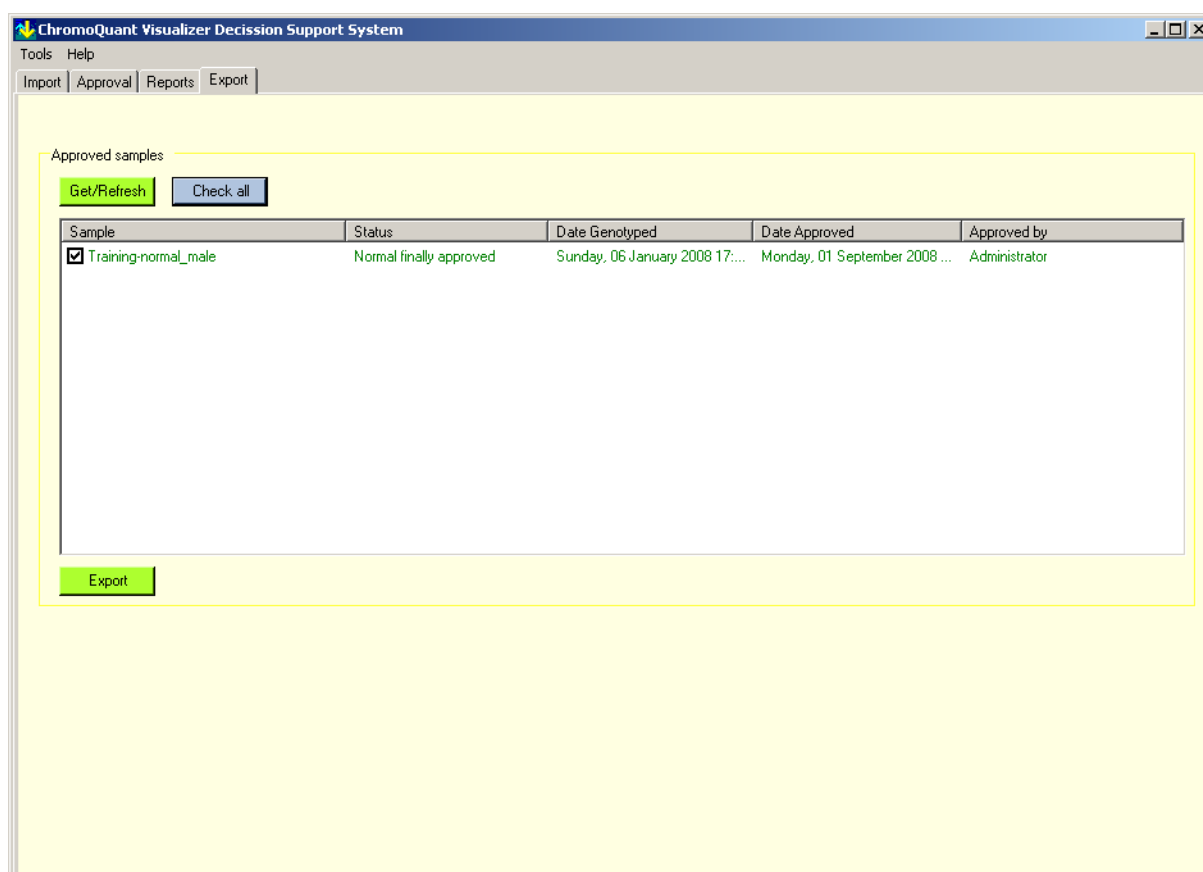
A print preview dialogue will appear where you can select appropriate printing options. The print preview dialogue will however vary depending on your Windows installation.



9 Exporting data

Visualizer data that has been approved can be exported as delimited text files. The purpose is to allow import of Visualizer data and analysis result into other LIM (Laboratory Information Systems). To configure your export settings please refer to the settings section.

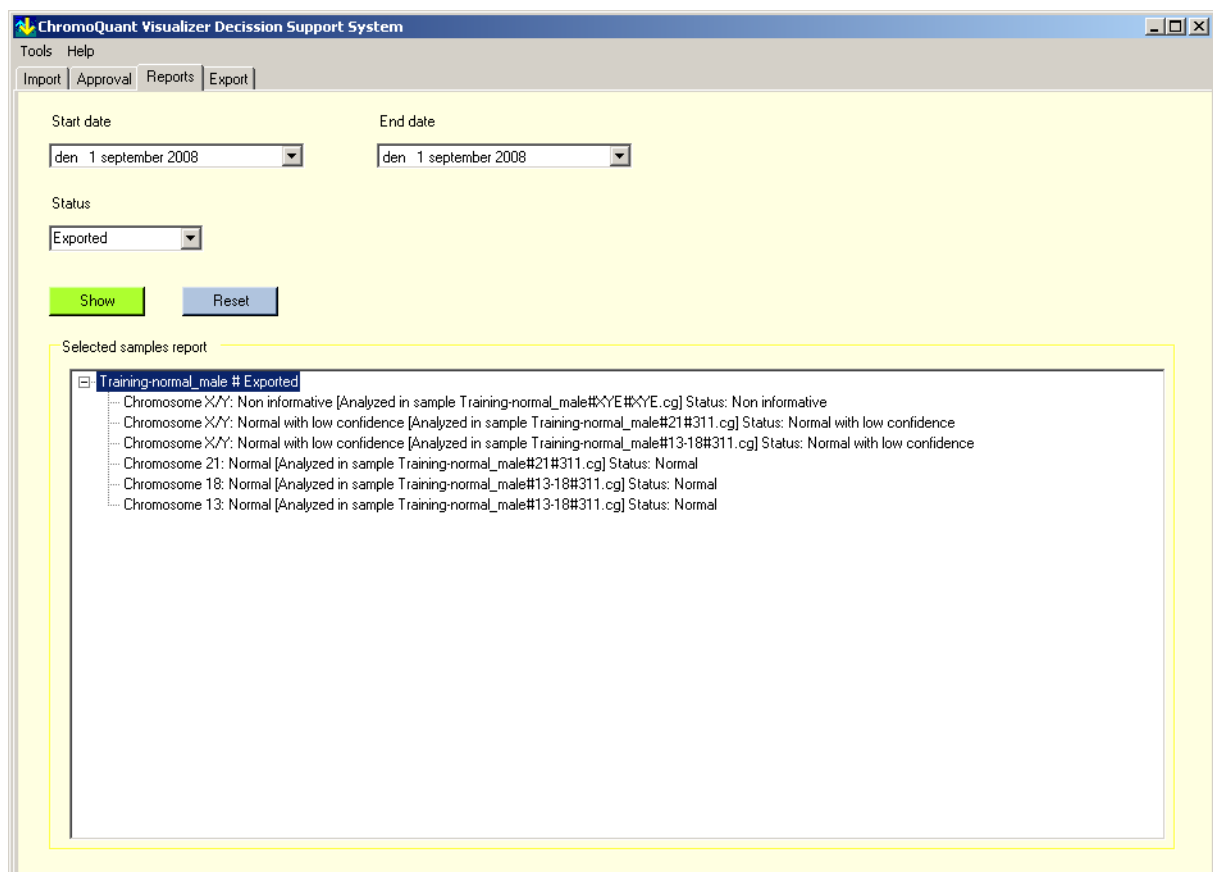
First of all you have to select the Export tab and thereafter click the *Get/Refresh* button. This will give a listing of all approved samples. You can click this button at any time to refresh the listing.



Select the samples you want to export. Clicking the *Check all* button will select all samples in the listing; individual samples can be checked and/or un-checked.

Clicking the *Export* button will effectuate the export. The export files will be saved in the destination directory you specified in your export setting. Likewise, delimiter and end of file characters will be in accordance to your settings.

Each exported sample will have its status changed to *Exported*. A timestamp will be set containing export date/time and name of user who performed the export. Exported samples can still be reviewed in the reports. However, its status cannot be changed nor can there be any additional comments added to it. For all practical purposes, exported samples are in their final state and cannot be altered.



Export files are named with a timestamp and an underscore followed by export for the mandatory export fields, exportComments and/or exportTimestamps depending on your settings. The export files have type txt as they are delimited text files.

10 Interpreting QF-PCR data

ChromoQuant user's guide and ChromoQuant Instructions for Use both contain information for how to best interpret ChromoQuant QF-PCR data. You can also direct questions or submit input by e-mail to chromoquant@cybergene.se